UNIVERSITY OF HELSINKI

https://helda.helsinki.fi

DPD-inspired discovery of novel LsrK kinase inhibitors: an opportunity to fight antimicrobial resistance

Stotani, Silvia; Gatta, Viviana; Medarametla, Prasanthi; Padmanaban, Mohan; Karawajzyk, Anna ...

2019-03-14
American Chemical Society
http://hdl.handle.net/10138/313322
Stotani, S, Gatta, V, Medarametla, P, Padmanaban, M, Karawajzyk, A, Giordanetto, F, Tammela, P, Laitinen, T, Poso, A, Tzalis, D & Collina, S 2019, 'DPD-inspired discovery of novel LsrK kinase inhibitors: an opportunity to fight antimicrobial resistance', Journal of Medicinal Chemistry, vol. 62, no. 5, pp. 2720-2737. https://doi.org/10.1021/acs.jmedchem.9b00025

Downloaded from Helda, University of Helsinki institutional repository. https://helda.helsinki.fi
This is an electronic reprint of the original article.
This reprint may differ from the original in pagination and typographic detail.
Please cite the original version.

DPD-inspired discovery of novel LsrK kinase inhibitors: an opportunity to fight antimicrobial resistance

Silvia Stotani[1,2], Viviana Gatta[3], Prasanthi Medarametla[4], Mohan Padmanaban[2], Anna Karawajczyk[5†], Fabrizio Giordanetto[2,§], Päivi Tammela[3], Tuomo Laitinen[4], Antti Poso[4], Dimitros Tzalis[2] and Simona Collina[2,]*

[1]Department of Drug Sciences, Medicinal Chemistry and Pharmaceutical Technology Section, University of Pavia, Viale Taramelli 12, 27100, Pavia, Italy

[2]Medicinal Chemistry, Taros Chemicals GmbH & Co. KG, Emil-Figge-Straße 76a, 44227 Dortmund, Germany

[3]Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland

[4]School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland

[5]Selvita S.A., Park Life Science, Bobrzyńskiego 14, 30-348 Krakow, Poland

KEYWORDS: Antibiotic resistance; Quorum Sensing; LsrK inhibition; DPD-related compounds

ABSTRACT: Antibiotic resistance is posing a continuous threat to global public health and represents a huge burden for society as a whole. In the last decade, the interference with bacterial Quorum

Sensing (QS) (i.e., cell-cell communication) mechanisms has extensively been investigated as a valid therapeutic approach in the pursuit of a next generation of antimicrobials. (*S*)-4,5-dihydroxy-2,3-pentanedione, commonly known as (*S*)-DPD, a small signaling molecule that modulates QS in both Gram-negative and Gram-positive bacteria, is phosphorylated by LsrK and the resulting phospho-DPD activates QS. We designed and prepared a small library of DPD derivatives, characterized by five different scaffolds and evaluated their LsrK inhibition in the context of QS interference. SAR studies highlighted the pyrazole moiety as an essential structural element for LsrK inhibition. Particularly, four compounds were found to be micromolar LsrK inhibitors (IC_{50} ranging between 100 μM and 500 μM) encouraging further exploration of novel analogues as potential new antimicrobials.

1. INTRODUCTION

Quorum Sensing (QS) is a cell-cell communication strategy that allows bacteria to act as a population by coordinating their gene expression.[1–5] QS regulates different pathogenic processes such as biofilm formation,[6–8] susceptibility to antibiotics[9] and virulence factor production. It is therefore not surprising that QS modulation has emerged in the last decade as a potential tool to fight antibiotic resistance.[10–13]

QS is mediated by the exchange of small signaling molecules termed autoinducers (AIs). Oligopeptides and *N*-acyl homoserine lactones (AHLs) are the most common QS mediators in Gram-positive and Gram-negative bacteria, respectively, while Autoinducer-2 (AI-2) features in both classes. Targeting AI-2-mediated QS would therefore result in broad spectrum antimicrobial activity. The precursor of AI-2, (*S*)-DPD, is biosynthesized intracellularly in a three steps pathway. *S*-adenosylmethionine (SAM) is demethylated by a methyltransferase to generate the toxic intermediate *S*-adenosylhomocysteine (SAH). In LuxS-containing organisms, the enzyme Pfs removes adenine from SAH to form *S*-ribosylhomocysteine (SRH). Lastly, LuxS breaks down SRH into adenine and (*S*)-DPD. LuxS synthase is found in more than 70 evolutionary different bacterial species indicating that AI-2 mediates both inter- and intraspecies communication.[14] The neo-synthetized (*S*)-DPD is released extracellularly with an unclear mechanism which might involve the membrane protein YdgG (Figure 1)[15,16]. Outside the cell membrane, linear (*S*)-DPD spontaneously rearranges into the two cyclic isomers *S*-DHMF and *R*-DHMF (Figure 1).[17] The aqueous environment allows for their hydration at C_3 to form the two cyclic tetrahydrated isomers *S*-THMF and *R*-THMF (Figure 1). X-ray crystallography revealed that *S*-*T*HMF, in the form of the borate ester *S*-THMF-borate, binds to the periplasmic protein LuxP[18] activating QS in *V. harveyi.* In enteric bacteria, when a threshold level of AI-2 in the extracellular medium is reached, *R*-THFM is imported via the Lsr (LuxS regulated) ACBFG transporter[19] and its linear form (i.e., S-THP) is phosphorylated by LsrK. The resulting S-THP-phosphate, commonly known as phospho-DPD (P-DPD, Figure 1), binds to the transcriptional repressor LsrR[20] that dissociates from the promoter

region of the *lsr* operon, initiating the operon transcription. As a result, the expression of the transporter on the cell surface is increased as well as the internalization of AI-2 and the QS response (Figure 1).[21] Ultimately, P-DPD is processed by LsrG and LsrF to close the AI-2 signaling cycle. LsrG catalyzes P-DPD isomerization to 3,4,4-trihydroxy-2-pentanone-5-phosphate (P-TPO, Figure 1)[22] while LsrF acts as a thiolase and transfers an acetyl group from the hydrated form of P-TPO to coenzyme A to form dihydroxyacetone phosphate (DHAP, Figure 1) and acetyl-CoA (Figure1).[22] Considering the complexity of the mechanism and its regulation, there is a great interest of the pharmaceutical community in identifying new compounds able to modulate QS to control bacterial virulence and reduce its negative effects, including mortality.[23–25] To lay the foundation for the rational design/discovery of QS modulators/inhibitors, several attempts to crystallize QS receptors have been performed. LuxP from *V. harveyi* has been co-crystallyzed with *S*-*T*HMF-borate (PDB ID: 1JX6[18]) while its isomer, *R*-THMF, has been solved in complex with the LsrB transporter (PDB ID: 1TJY[19]). Lastly, the linear hydrated form of (*S*)-DPD has been shown to bind to the transcriptional repressor LsrR (PDB ID: 4L4Z[20]).

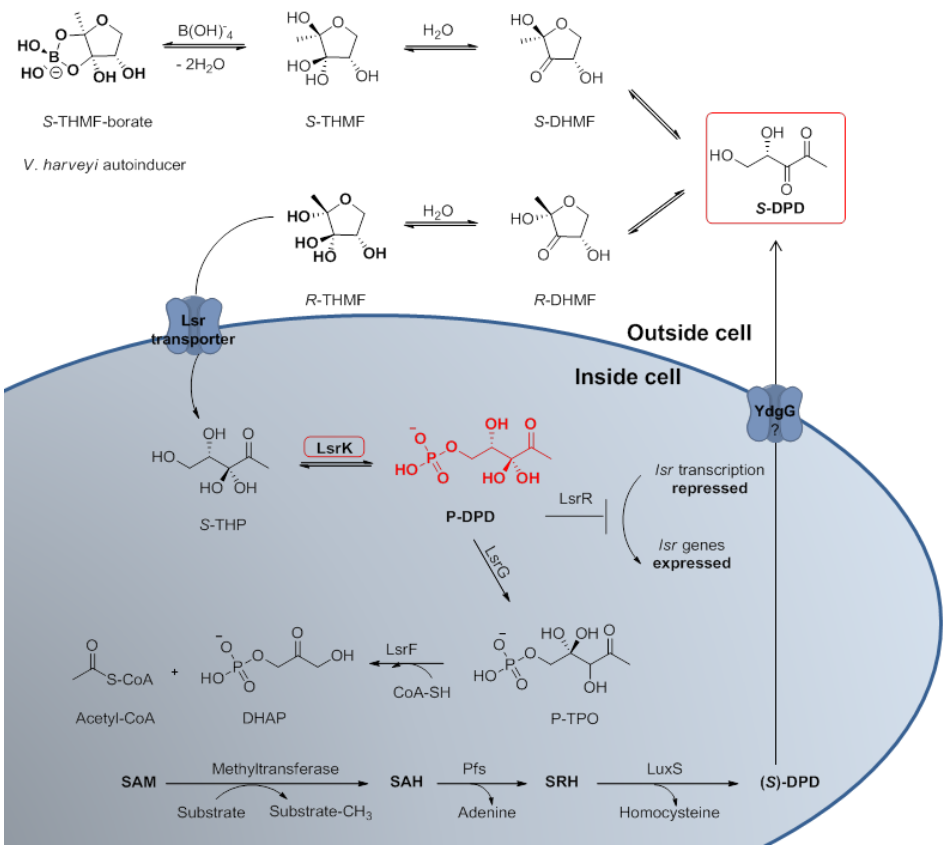


Figure 1: Biosynthesis, transport and degradation of (*S*)-DPD in enteric bacteria

To date, several structure-activity relationship (SAR) studies have been carried out around the DPD backbone and the effects of substitution at the C_1 position have been reported.[26–33] However, the exploration of new compound classes that are structurally distinctive from native DPD is needed to discover new antibacterial agents. In this study, we report on the design, synthesis and SAR of novel DPD-related compounds (Figure 2) with different "core structures", involving modifications at the DPD diketo moiety. In particular, we explored five small libraries of DPD-inspired heterocyclic derivatives (Het-DPD derivatives, Figure 2). Lastly, the *in vitro* inhibitory effect of the new compounds against LsrK has been evaluated. Results obtained suggested that Het-DPD derivatives act as LsrK inhibitors and their analogs may be considered as useful templates for the discovery of new effective DPD-based antimicrobial agents. To the best of our knowledge, these are the first heterocyclic-based LsrK inhibitors reported to-date.

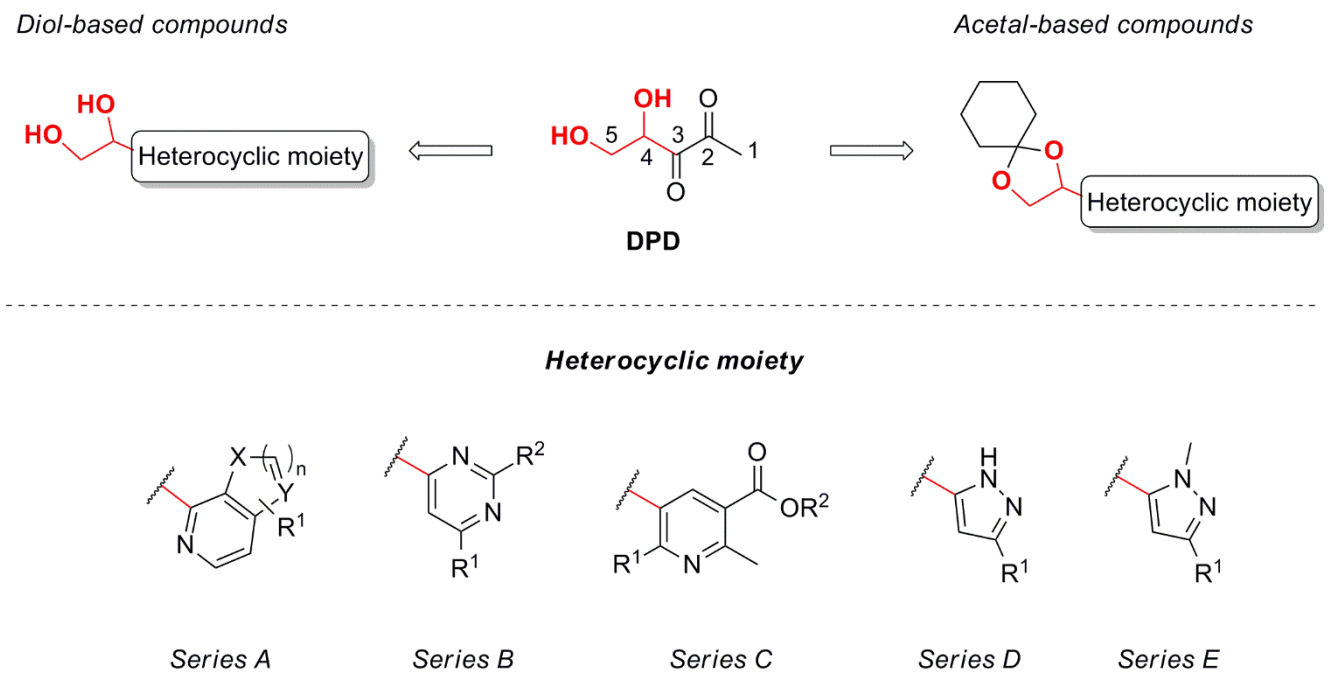


Figure 2: DPD-inspired heterocyclic compounds

2. RESULTS AND DISCUSSION

2.1 Compounds design

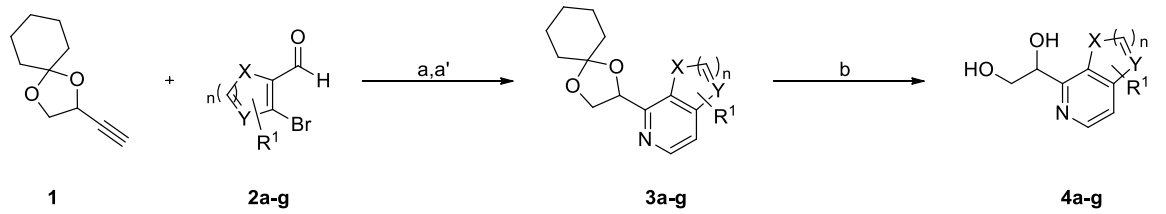
LsrK belongs to a family of carbohydrate kinases called "FGGY family" whose members transfer a phosphate group from ATP to several sugars ranging from trioses to heptoses.[34] In enteric bacteria, phosphorylation of DPD by LsrK represents the prerequisite activating step for AI-2 signaling. Modulation/inhibition of LsrK could therefore potentially attenuate AI-2-related pathogenesis and LsrK could be a new and attractive anti-infective target to further explore.

Previous studies evidenced that both the hydroxyl groups at C_4 and C_5 of DPD (Figure 2) are essential for phosphorylation. It has been demonstrated that DPD is phosphorylated at position five and that phosphorylation occurs only when the hydroxyl group at C_4 is not derivatized.[35] Starting from these considerations, we herein designed new DPD-related compounds where the portions essential for LsrK-mediated phosphorylation (i.e., the two hydroxyl groups at C_4 and C_5) are kept constant while the

diketo moiety of DPD is embedded in heteroaromatic rings. The strategy of incorporating the carbonyl groups at C2 and C3 in heteroaromatic moiety may allow to obtain compounds stable in solution, and to avoid the open/closed equilibrium typical of the majority of the DPD-analogs reported so far. e focused our attention on different heterocyclic scaffolds decorated with different aliphatic and/or aromatic moieties (Schemes 1 – 4), with the final aim to investigate the chemical space of compounds structurally related to DPD. Among the wide variety of heterocycles, we selected annulated pyridines, pyridines, pyrimidines and pyrazoles, given their high frequency of occurrence in several natural or synthetic drugs used for their antibacterial[36,37], antifungal[38–41] anticancer[42–45] and anti-inflammatory[46–48] properties, just to cite a few.

2.2 Synthesis

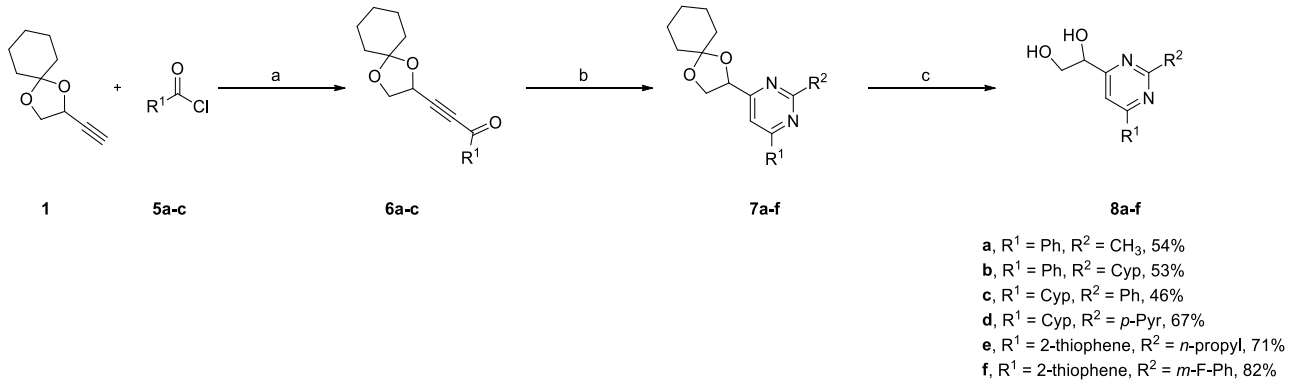
Target compounds (Schemes 1 – 4) can be easily prepared applying our recently developed protocol, starting from intermediate **1** bearing an ethyne group, suitable for further chemical diversification.[49] The synthesis of monosubstituted annulated pyridines-Het-DPD derivatives (Figure 2, **series A**) was accomplished *via* a one pot, microwave-assisted, palladium-catalyzed strategy, coupling alkyne **1** with *o*-bromoaldehydes. The resulting Sonogashira products were reacted with ammonium acetate as imination reagent and then deprotected (Scheme 1). Briefly, alkyne **1** was dissolved in DMF and then $Pd(OAc)_2$, PPh_3, KOAc and the appropriate *o*-bromoaldehyde were added. The reaction mixtures were irradiated at 80 °C for 1 – 2 hours, ammonium acetate was added and the resulting mixtures were irradiated again at 150 °C, thus affording the desired protected products **3a-g** (Scheme 1). Heteroaromatic *o*-bromoaldehydes (i.e., 3-bromofuran-2-carbaldehyde **2e**, 3-bromothiophene-2-carbaldehyde **2f** and 5-bromo-2-methyl-4-thiazolecarboxaldehyde **2g**) led to isolation of the corresponding furopyridine, thienopyridine and thiazolopyridine derivatives **3e-f**. Final acidic treatment yielded the monosubstituted annulated pyridines-Het-DPD derivatives **4a-g** (Scheme 1).



a, n = 2, X = H, Y = H, R^1 = H, 90%
b, n = 2, X = H, Y = H, R^1 = *p*-CH_3, 54%
c, n = 2, X = H, Y = H, R^1 = *m*-OH, 67%
d, n = 2, X = H, Y = H, R^1 = *o*-F, 42%
e, n = 1, X = O, Y = H, R^1 = H, 48%
f, n = 1, X = S, Y = H, R^1 = H, 73%
g, n = 1, X = S, Y = N, R^1 = CH_3, 70%

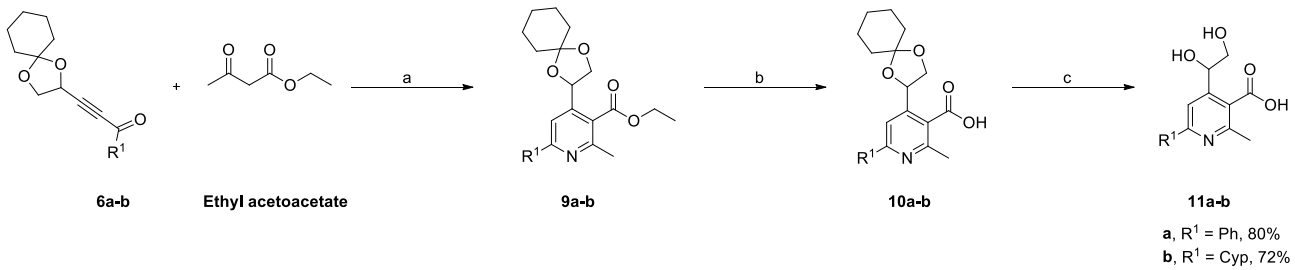
Scheme 1: Synthesis of monosubstituted annulated pyridines-Het-DPD derivatives **4a-g**. Reagents and conditions: (a) **2a-g** (0.9 eq), $Pd(OAc)_2$ (1.8% mol), PPh_3 (3.6% mol), KOAc (1.8 eq), DMF, mw, 80 °C, 1h – 2h; (a') NH_4OAc (1.8 eq), MW, 150 °C, 2h – 3h; (b) 12M HCl (cat.), 1,4-dioxane, 0 °C to rt, 1h – 3h

Sonogashira coupling[43] of terminal alkyne **1** with acyl chlorides, followed by addition of amidinium salts to the corresponding ynones and final acidic removal of the acetal protecting group provided rapid access to 2,4,6-trisubstituted pyrimidines-Het-DPD derivatives (Figure 2, **series B**). Benzoyl chloride **5a** was selected to screen three different conditions for the synthesis of ynone **6a**. Surprisingly, copper-, ligand- and solvent-free acylation[51] as well as the use of a mixture of $PdCl_2(CH_3CN)_2$, Sphos and Cs_2CO_3[26] did not furnish the desired product. Good results were obtained with the copper- and palladium-catalyzed system proposed by Karpov *et al.*[50] which allowed us to obtain compound **6a** with a yield of 87%.[52] Three different ynones (**6a-c**) were reacted with six different amidinium salts and the resulting products **7a-f** treated under acidic conditions (i.e., Scheme 2, conditions c) to afford the 2,4,6-trisubstituted pyrimidines-Het-DPD derivatives **8a-f** with moderate to excellent yields (i.e., 43% – 82%).



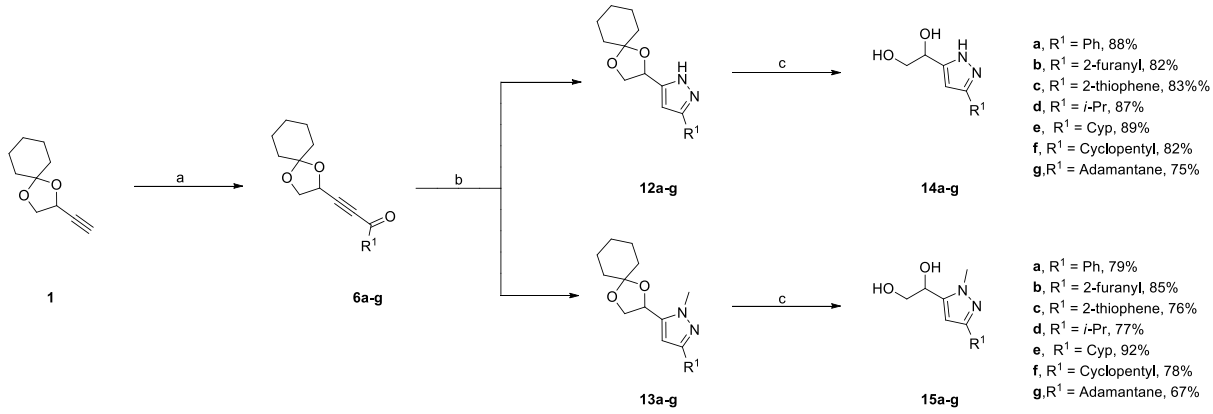
Scheme 2: Synthesis of 2,4,6-trisubstituted pyrimidines-Het-DPD derivatives **8a-f**. Reagents and conditions: (a) **5a-c** (1.5 eq), $PdCl_2(PPh_3)_2$ (9% mol), CuI (3% mol), Et_3N (1.25 eq), THF, rt, overnight; (b) R_2NHNH_2*HCl (1.2 eq), Na_2CO_3*10 H_2O (3.0 eq), 40 °C, overnight; (c) 12M HCl (cat.), 1,4-dioxane, 0 °C to rt, 1h – 3h

The 2,3,4,6-tetrasubstituted pyridines-Het-DPD derivatives (Figure 2, **series C**) were easily obtained following the one-pot, three-component and acid-free methodology developed by Bagley *et al.* This heteroannulation combines a Michael acceptor with a nucleophile in the presence of an excess (10.0 eq) of ammonium acetate (to generate, *in situ*, the enamine).[53] Ethyl acetoacetate was selected as nucleophile to have an additional diversification point (i.e., the carboxylic ester moiety) and reaction with **6a** (i.e., the Michael acceptor) in refluxing ethanol provided intermediate **9a** with 92% yield (Scheme 3). Tetrasubstituted pyridines **9a** and **9b** (from ynone **6b**), were hydrolyzed in basic conditions and then treated with hydrochloric acid to give target compounds **11a-b** with excellent yields (i.e., 80% and 72%, respectively).



Scheme 3: Synthesis of 2,3,4,6-tetrasubstituted pyridines-Het-DPD derivatives **11a-b**. Reagents and conditions: (a) Ethyl acetoacetate (10.0 eq), EtOH, 50 °C, overnight; (b) 1M NaOH (3.0 eq), EtOH, 0 °C to rt, overnight; (c) 12M HCl (cat.), 1,4-dioxane, 0 °C to rt, 1h – 3h

The synthesis of 3,5-disubstituted (**series D**) and 1,3,-5-trisubstituted pyrazoles-Het-DPD derivatives (**series E**) started with the production of three additional aliphatic (i.e., isopropyl, cyclopentyl and adamantane **6d-f**) and one aromatic (i.e., furanyl **6g**) ynones. Each of the seven ynones (**6a-g**) was reacted with two different hydrazines (i.e., hydrazine and methylhydrazine) to generate, respectively, 3,5-disubstituted (**12a-g**) and 1,3,5-trisubstituted (**13a-g**) protected pyrazoles. Final acidic deprotection afforded the targeted products **14a-g** and **15a-g** with excellent yields (i.e., 67% – 92%, Scheme 4).



Scheme 4: Synthesis of 3,5-disubstituted and 1,3,5-trisubstitted pyrazoles-Het-DPD derivatives **14a-g** and **15a-g**. Reagents and conditions: (a) R_1COCl (1.5 eq), $PdCl_2(PPh_3)_2$ (9% mol), CuI (3% mol), Et_3N (1.25 eq), THF, rt, overnight; (b) R_2NHNH_2 (1.3 eq), EtOH, rt, overnight; (c) 12M HCl (cat.), 1,4-dioxane, 0 °C to rt, 1h – 3h

2.3 Screening of LsrK inhibitors

All the prepared compounds were assayed for inhibitory activity against LsrK at 200 μM concentration. Unexpectedly, only four compounds (belonging to **series D** and **series E**) among the 29 prepared, resulted effective in inhibiting LrsK. Compounds **12a**, **12b** (**series D**) and **13a**, **13b** (**series E**) showing an inhibition percentage higher than 40%, were retested at different concentrations (Table 1) and their IC_{50} values calculated. In Figure 3 the dose-response curves together with the IC_{50} values of compounds **12a**, **12b**, **13a**, **13b** are reported.

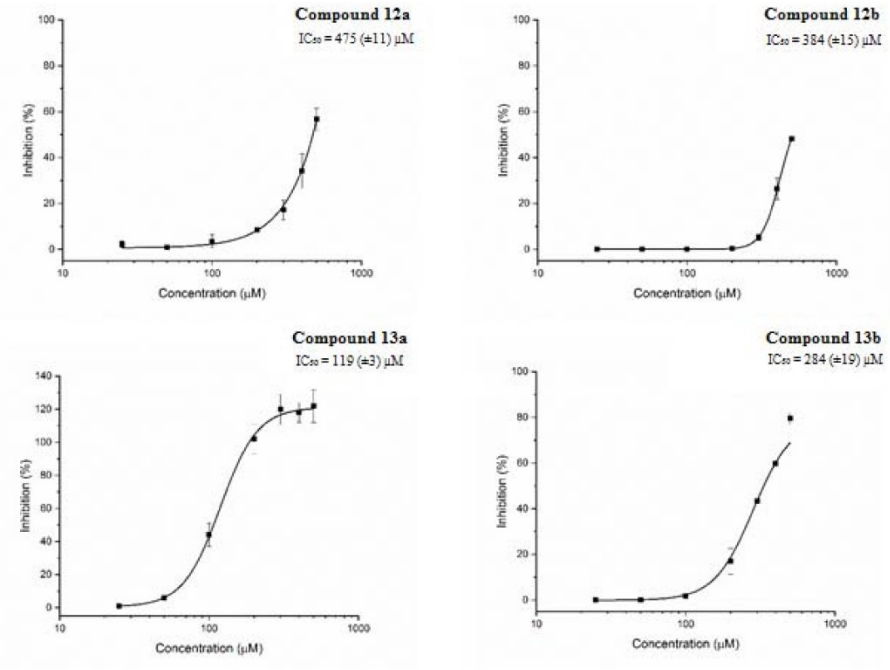
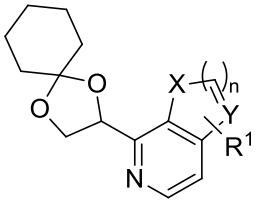
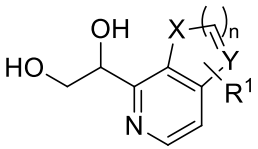
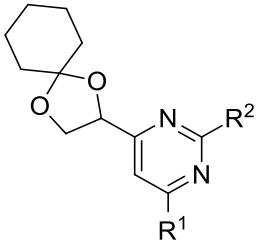
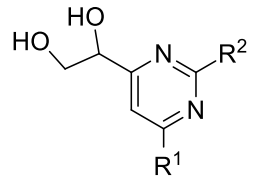
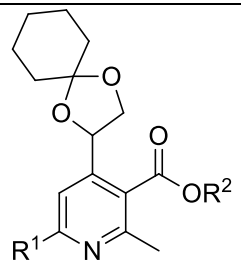
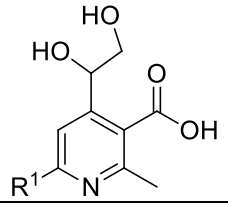
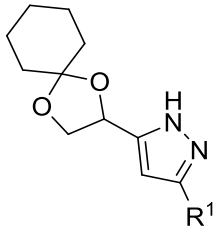
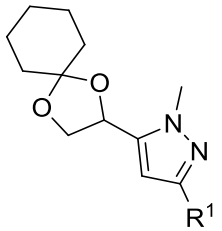
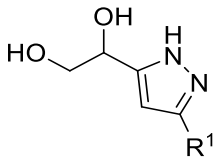
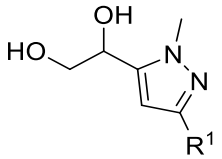


Figure 3: Dose-response curves against LsrK for compounds **12a**, **12b**, **13a**, **13b**. Data points represent the mean ±SD of two independent experiments (n = 4)

To evaluate the specificity of compounds **12a**, **12b**, **13a**, **13b**, these were further tested against glycerokinase, which has high sequence similarity (i.e., 41.2%) to LsrK. None of the compounds showed activity against glycerokinase (Figure S1, SI). To sum up, pyrazole-containing DPD derivatives emerged as the most interesting LsrK inhibitors among the series under investigation.

Series	Structure	Compound	R^1	R^2	X	Y	N	Inhibition (%)	SD
Annulated pyridines and derivatives **Series A**		**3a**	H	—	H	H	H	14.91	1.53
		3b	*p*-CH_3		H	H	2	1.66	1.22
		3c	*m*-OH		H	H	2	28.55	3.83
		3d	*o*-F		H	H	2	1.76	0.94
		3e	H		O	H	1	5.75	1.41
		3f	H		S	H	1	11.06	4.02
		3g	CH_3		S	N	1	6.32	2.35
Annulated pyridines and derivatives **Series A**		**4a**	H	—	H	H	2	0.48	0.63
		4b	*p*-CH_3		H	H	2	0.40	0.39
		4c	*m*-OH		H	H	2	1.67	1.55
		4d	*o*-F		H	H	2	0.65	0.41
		4e	H		O	H	1	2.07	1.00
		4f	H		S	H	1	0.17	0.42
		4g	CH_3		S	N	1	0.10	0.42
2,4,6-trisubstituted pyrimidines **Series B**		**7a**	Ph	CH_3	—	—	—	9.41	1.41
		7b	Ph	Cyp				15.18	4.39
		7c	Cyp	Ph				0.76	0.74
		7d	Cyp	*p*-Pyr				21.22	3.23
		7e	2-thiophene	*n*-propyl				0.96	0.85
		7f	2-thiophene	*m*-F-Ph				0.20	0.33
2,4,6-trisubstituted pyrimidines **Series B**		**8a**	Ph	CH_3	—	—	—	0.22	0.69
		8b	Ph	Cyp				24.48	3.32
		8c	Cyp	Ph				21.65	2.37
		8d	Cyp	*p*-Pyr				2.94	1.11
		8e	2-thiophene	*n*-propyl				47.76	2.94
		8f	2-thiophene	*m*-F-Ph				19.79	1.42

Series	Structure	Compound	R^1	R^2	X	Y	N	Inhibition (%)	SD
2,3,4,6-tetrasubstituted pyridines **Series C**		**9a**	Ph	CH_3CH_2	—	—	—	0.10	0.07
		9b	Cyp	CH_3CH_2				0.36	0.66
		10a	Ph	H				0	1.69
		10b	Cyp	H				6.26	0.65
2,3,4,6-tetrasubstituted pyridines **Series C**		**11a**	Ph		—	—	—	0.81	0.59
		11b	Cyp					1.33	0.49
3,5-disubstituted pyrazoles **Series D**		**12a**	Ph		—	—	—	53.60	5.67
		12b	2-furanyl					43.48	26.98
		12c	2-thiophene					26.50	5.52
		12d	*i*-Pr					7.14	1.78
		12e	Cyp					1.47	0.26
		12f	Cyclopentyl					0	0.21
		12g	Adamantane					0	0.81

Series	Structure	Compound	R^1	R^2	X	Y	N	Inhibition (%)	SD
1,3,5-trisubstitted pyrazoles **Series E**		**13a**	Ph	—	—	—	—	78.05	3.44
		13b	2-furanyl					51.63	11.91
		13c	2-thiophene					0	0.22
		13d	*i*-Pr					0	0.67
		13e	Cyp					0	0.57
		13f	Cyclopentyl					0	0.82
		13g	Adamantane					0	0.11
		13h	*m*-CH_3-Ph					1.07	0.91
		13i	*m*-CN-Ph					0	0.51
		13j	*m*-Cl-,*m*-F-Ph					4.42	2.14
		13k	*p*-$N(CH_3)_2$-Ph					0.19	1.11
		13l	3-isoxazole					9.83	1.74
		13m	2-indole					4.38	2.39
		13n	*m*-Pyr					4.92	5.13
		13o	CyHex					1.51	1.50
3,5-disubstituted pyrazoles **Series D**		**14a**	Ph	—	—	—	—	0	0.02
		14b	2-furanyl					0	0.05
		14c	2-thiophene					0	1.16
		14d	*i*-Pr					0	0.06
		14e	Cyp					0	0.08
		14f	Cyclopentyl					0	0.96
		14g	Adamantane					0	0.44
1,3,5-trisubstitted pyrazoles **Series E**		**15a**	Ph	—	—	—	—	0	0.46
		15b	2-furanyl					0	0.66
		15c	2-thiophene					0	0.05
		15d	*i*-Pr					0	0.24
		15e	Cyp					0	0.47
		15f	Cyclopentyl					0	0.32
		15g	Adamantane					0	0.72

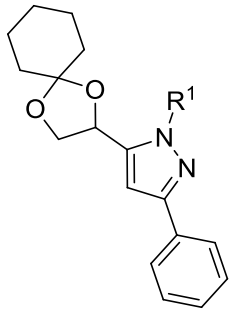
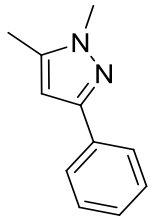
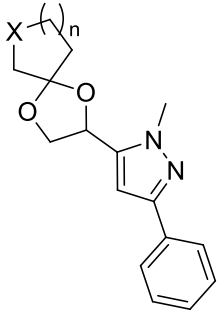
Series	Structure	Compound	R^1	R^2	X	Y	N	Inhibition (%)	SD
3,5-disubstituted pyrazoles **New pyrazole derivatives**		**16a**	CH_3CH_2	—	—	—	—	0	0.62
		16b	*i*-Pr					0	0.96
		16c	Cyp					0	0.70
		16d	$(CH_2)_2$-CN					0	0.83
		16e	Cyclopentyl					0	0.59
		16f	Ph					0	0.53
3,5-disubstituted pyrazoles **New pyrazole derivatives**		**18**	—	—	—	—	—	2.01	0.78
3,5-disubstituted pyrazoles **New pyrazole derivatives**		**22a**	—	—	H	—	0	5.56	2.34
		22b			H		2	0	0.64
		22c			O		1	31.13	1.83
		22d			N-Ac		1	2.07	1.65

Table 1: Activities of the synthesized DPD-IHs

2. Structure-Activity Relationship (SAR) studies of pyrazole-containing DPD derivatives

The results of the first LsrK screening highlighted the pyrazole moiety as the most promising scaffold. Accordingly, we further explored the 1,3,5-trisubstituted pyrazole series (**series E**), applying our synthetic protocol which permits an easy access to molecular diversity at three different diversification points, as outlined in Figure 4. Based on synthetic feasibility and commercial availability of the starting materials, nineteen new 1,3,5-trisubstituted pyrazole derivatives have been prepared by installing different aliphatic and aromatic substituents at position one (Scheme 5) and three (Scheme 6) as well as by replacing at position five the cyclohexylidene protecting group with aliphatic rings of different sizes (Scheme 7). Moreover, the dioxolane ring was removed (compound 18, Scheme 7).

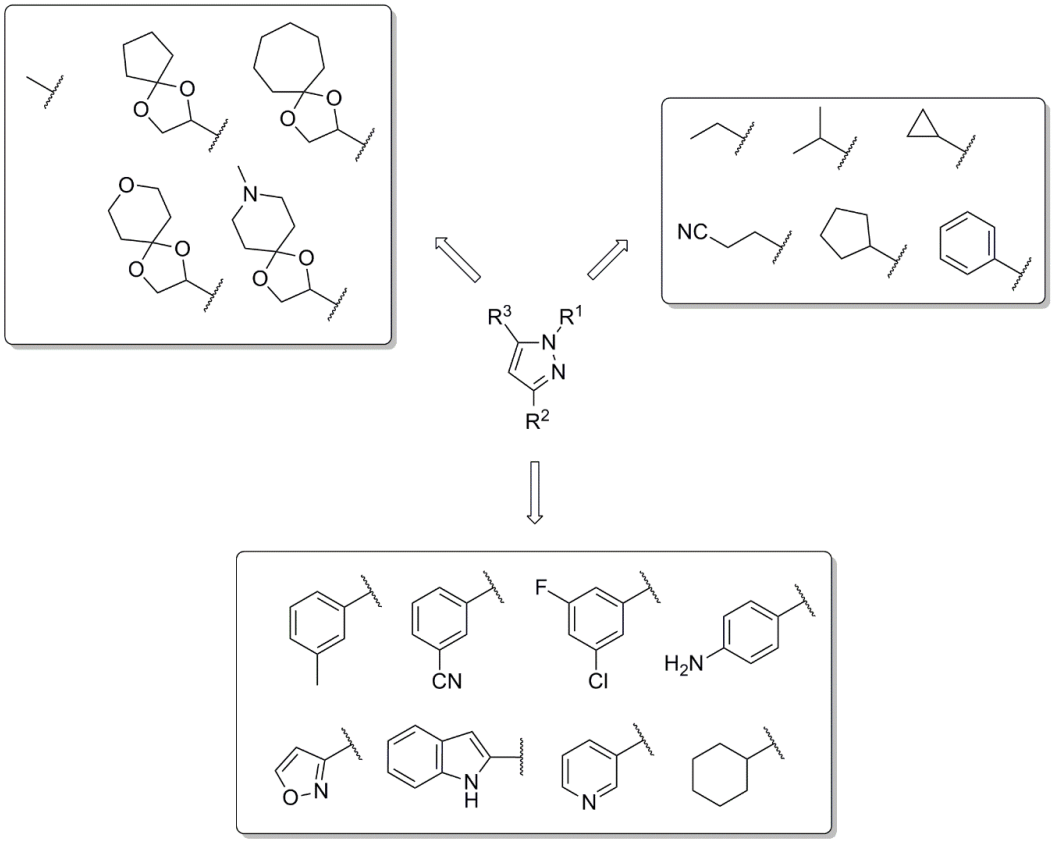
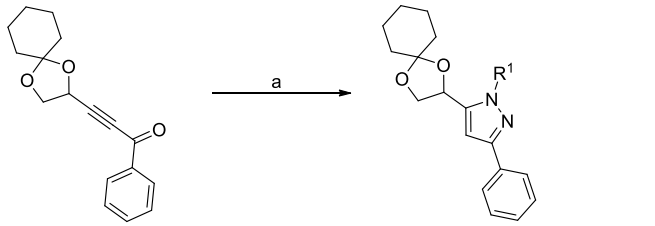


Figure 4: Further diversification of 1,3,5-trisubstituted pyrazolic compounds

For modification at N_1 the effects of aliphatic (linear, branched and cyclic) and aromatic substituents on bioactivity were investigated. The synthesis of this second set of 1,3,5-trisubstituted pyrazoles-Het-DPD derivatives (**16a-f**) started from ynone **6a** to which was added an excess (1.3 eq) of the corresponding hydrazine.

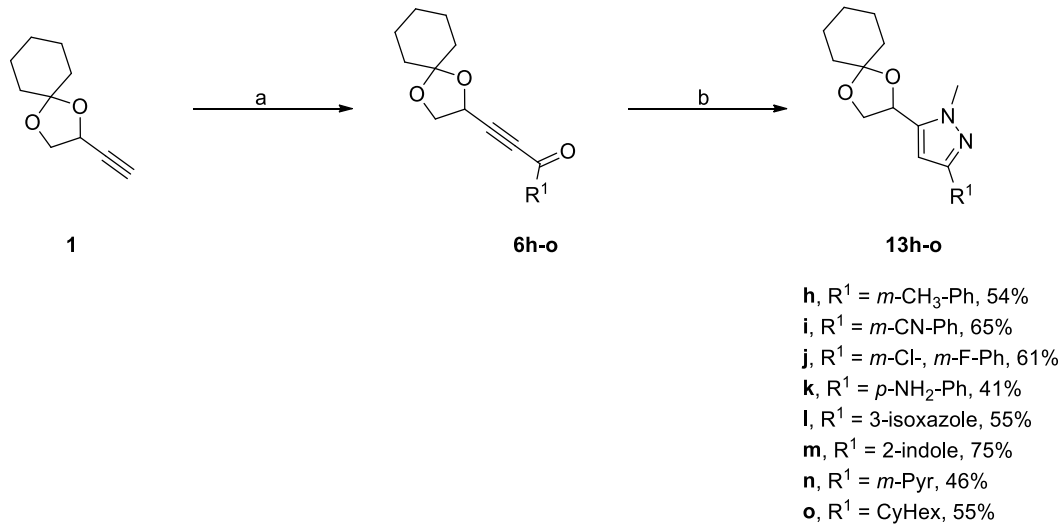


6a **16a-f**

a, R^1 = Et, 44%
b, R^1 = *i*-Pr, 60%
c, R^1 = Cyp, 54%
d, R^1 = $(CH_2)_2$-CN, 41%
e, R^1 = Cyclopentyl, 70%
f, R^1 = Ph, 55%

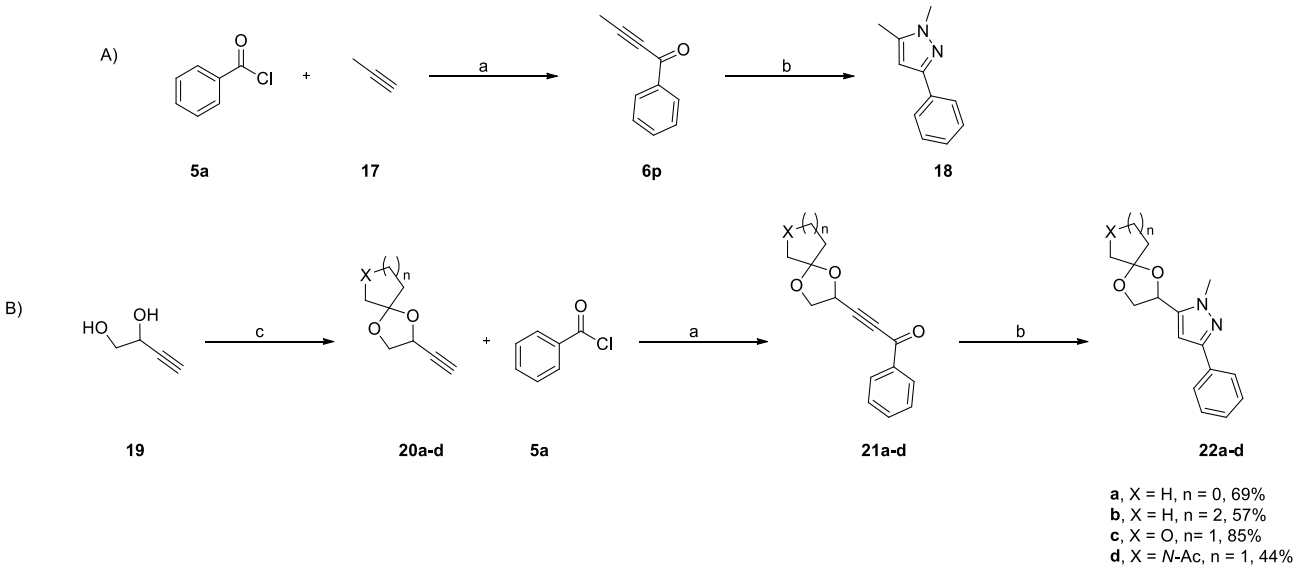
Scheme 5: Synthesis of the first new set of 1,3,5-trisubstituted pyrazoles-Het-DPD derivatives **16a-f**. Reagents and conditions: (a) R_1NHNH_2 (1.3 eq), EtOH, rt, overnight

Several analogues where the phenyl at position three of the pyrazole nucleus was replaced with other aliphatic, aromatic and heteroaromatic rings were then studied. Compounds **16h-o** (Scheme 6) were readily prepared by condensation of eight new ynones synthetized by us with methylhydrazine.



Scheme 6: Synthesis of the second new set of 1,3,5-trisubstituted pyrazoles-Het-DPD derivatives **13h-o**. Reagents and conditions: (a) R_1COCl (1.5 eq), $PdCl_2(PPh_3)_2$ (9% mol), CuI (3% mol), Et_3N (1.25 eq), THF, rt, overnight; (b) $MeNHNH_2$ (1.3 eq), EtOH, rt, overnight

Lastly, to study the relevance of the dioxolane ring on biological activity, we prepared compound **18**, where it was completely removed. Additionally, derivatives **22a-d**, bearing acetal substituents with different steric properties were also synthesized. Compound **18** was prepared reacting benzoyl chloride **5a** with 1-propyne **17** and the resulting ynone **6p** with methylhydrazine as previously described (Scheme 7A). By reaction of the diol **19** with an excess (3.0 eq) of the corresponding ketone and a catalytic amount of *p*-TSA in neat conditions, further acylation with **5a** and condensation with methylhydrazine the desired five new pyrazoles-Het-DPD **22a-d** (Scheme 7B) were obtained. It has to be noted that 4-piperidone was found not to be suitable for our purpose as the free basic nitrogen impeded the Sonogashira coupling with benzoyl chloride while *N*-acetylpiperidone did not show any reactivity issue and compound **22d** could be isolated with 44% yield.



Scheme 7: A) Synthesis of compound **18**. Reagents and conditions: (a) **5a** (1.5 eq), 1-propyne (1.0 eq), $PdCl_2(PPh_3)_2$ (9% mol), CuI (3% mol), Et_3N (1.25 eq), THF, rt, overnight; (b) $MeNHNH_2$ (1.3 eq), EtOH, rt, overnight. B) Synthesis of the third new set of 1,3,5-trisubstituted pyrazoles-Het-DPD derivatives **22a-d**. Reagents and conditions: (c) Ketone (3.0 eq), *p*-TSA (cat.), rt, overnight

All the prepared 1,3,5-trisubstituted pyrazoles-Het-DPD were assayed for inhibitory activity against LsrK at 200 μM concentration, according to the protocol previously described.[54] The obtained results confirmed that the presence of the dioxolane ring at position 5 of the pyrazole moiety is essential for the binding to LsrK and that acetals derivatives have a better profile respect to the corresponding diols. Overall, the results indicate that the most active LsrK inhibitor is compound **13a**, bearing a methyl group at N_1, a phenyl group at C_3 and an acetal moiety at position five.

2.5 Molecular modeling studies

To support the SAR analysis and to inspect the molecular basis of their binding to LsrK, all the compounds were docked into the binding site of the LsrK crystal structure (PDB ID: 5YA1) using Glide, a molecular docking program reliable for predicting the binding mode of small molecules in

protein complexes. Hence, docking poses were analyzed for both interactions and geometry. At first, we considered the modification at the diol moiety of DPD, our inspiring molecule, since *in vitro* experiments suggested the presence of the dioxolane ring in the Het-DPD derivatives as essential for the binding to LsrK. We observed that the non-polar cyclohexane ring of acetal compounds can form electrostatic interactions with the surrounding environment. Compounds bearing the diol moiety showed an opposite orientation in the binding site (Figure S4, SI). The orientation of the diol groups towards the negative electrostatic potential surface of the binding site (Glu 454 and Thr 456) resulted in repulsion effects, thus explaining the weaker binding of diols compared with acetals.

We then focused on pyrazole Het-DPD derivatives (**series D** and **series E**) since the *in vitro* results of the first LsrK screening highlighted the pyrazole moiety as the most promising scaffold. In line with experimental data, compound **13a** gave the best interactions. Although compounds **12a** and **13a**, which differ only in the presence of N_1-CH_3, showed similar binding poses, we observed that the most active inhibitor **13a** formed a hydrogen bond interaction with Thr 275, whereas **12a** with Phe 276 and Gln 278 (Figure S2, SI). Hence, we speculated that the interaction with Thr 275 may be involved in the substrate phosphorylation reaction. The binding poses of **12a** and **13a** (Figure S3, SI and Figure 5) are similar to those of the less active furanyl-substituted derivatives **12b** and **13b**, with the only exception of the "pyrazole ring flipping", caused by the bulky N_1-CH_3 group. The conformation of the pyrazole in **12a** and **13a** resulted in a better methyl stabilization energy of N_1-CH_3, thus favoring the interaction with the target. Moreover, the oxygen of the furan ring of **13b** (Figure 5) originated a negative electrostatic potential and the local binding site environment, for example the Thr 456 residue contributed to the electrostatic repulsion.

Starting from these observations, further Het-DPD derivatives potentially able to better interact with LsrK will be designed and synthetized in the next future.

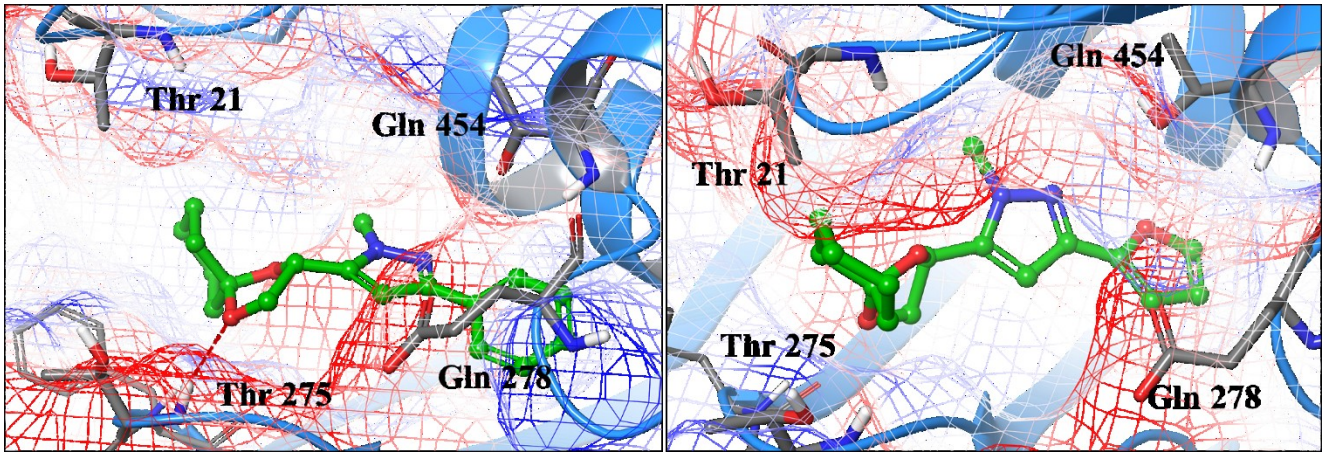


Figure 5. Compound **13a** (left) and **13b** (right) binding pose in the LsrK binding site (PDB ID: 5YA1). Hydrogen bond interactions (red dashed line) are shown along with active site residues. Electrostatic potential surfaces were presented as mesh (blue-positive potential and red-negative potential)

3. CONCLUSIONS

Resistance to antibiotics is constantly increasing and it is estimated that, by 2050, multidrug resistant bacteria will kill more people than cancer.[55] In the last decade, modulation of QS has become an attractive therapeutic strategy to fight bacterial resistance. Two different DPD-related compounds (i.e., isobutyl-DPD and phenyl-DPD) have already shown their activity in combination with gentamicin and small molecules able to modulate/inhibit QS are therefore considered interesting "non-conventional" tools to be used in combination with classical antibiotics.[10–13]

As a continuation of our recent findings, where, applying a virtual screening approach we were able to identify 2-substituted amino benzoic acids as LsrK inhibitors, in this communication, we report for the first time on the "*ad hoc*" design and synthesis of potential LrsK inhibitors structurally related to DPD. The medicinal chemistry efforts presented in this work provided 48 Het-DPD derivatives, characterized by different heterocyclic scaffolds and led to the identification of effective representatives characterized by *in vitro* LsrK enzymatic activity (IC_{50}) in the micromolar range. All the target

compounds have been easily prepared in maximum four synthetic steps, thus allowing to easily explore the chemical space of DPD. Remarkably, four compounds displayed IC_{50} values between 100 μM and 500 μM, evidencing the 3,5-disubstituted and 1,3,5-trisubstituted pyrazole structures as the best scaffolds. Moreover, an in-depth SAR campaign was performed, synthetizing other nineteen 1,3,5-trisubstituted pyrazole analogues. The systematic ligand-based approach and SAR studies, led to the identification of compound **13a**, with an IC_{50} value of 119 μM as a promising *Hit* for future chemical development of novel LsrK inhibitors. Future work in our laboratory will focus on a hit-to-lead process to increase LsrK inhibition by further diversification and functionalization of the heterocyclic core and the evaluation of these novel derivatives for their potential as QS-interfering compounds in the treatment of bacterial infections.

4. EXPERIMENTAL SECTION

4.1 Chemistry

Chemicals and solvents were obtained from commercial suppliers and were used without further purification. All dry reactions were performed under nitrogen atmosphere using commercial dry solvents. Flash column chromatography was performed on a silica column using 230400 mesh silica gel or Grace Reveleris X2 flash chromatography system using silica gel packed Macherey Nagel Chromabond Flash BT cartridges (60 Å, 45 μm) and Grace Reveleris flash Cartridges (60 Å, 40 μm). Thin layer chromatography was performed on Macherey Nagel precoated TLC aluminum sheets with silica gel 60 UV254 (5 μm – 17 μm). TLC visualization was accomplished by irradiation with a UV lamp (254 nm) and/or staining with $KMnO_4$ solutions. ^1H NMR spectra were recorded at room temperature on a Bruker Avance spectrometer operating at 300 MHz. Chemical shifts are given in ppm (δ) from tetramethylsilane as an internal standard or residual solvent peak. Significant ^1H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; td, triplet of doublets; br, broad), coupling constant(s) in hertz, number of protons. Proton decoupled ^{13}C NMR data were acquired at 100 MHz. ^{13}C chemical shifts are reported in parts per million (δ, ppm). All NMR data were collected at room temperature (25 °C). Analytical, preparative HPLC and Electron Spray Ionization (ESI) mass spectra were performed on an Agilent UHPLC (1290 Infinity) and an Agilent Prep-HPLC (1260 Infinity) both equipped with a Diode Array Detector and a Quadrupole MS using mixture gradients of formic acid/water/acetonitrile as solvents. High-resolution electrospray ionization mass spectra (ESI-FTMS) were recorded on a Thermo LTQ Orbitrap (high-resolution mass spectrometer from Thermo Electron) coupled to an ‘Accela’ HPLC system supplied with a ‘Hypersil GOLD’ column (Termo Electron). All

the intermediates and final compounds displayed ≥ 95.0% purity as determined by UHPLC (Agilent, 1290 Infinity). For each compound, the retention rime (R_t) is expressed in minutes (min.).

4.2 General procedures for the synthesis of monosubstituted annulated pyridines and derivatives (Series A)

Synthesis of 3a-g: 1 (1.0eq), $Pd(OAc)_2$ (1.8 %mol), PPh_3 (3.6 % mol), KOAc (1.8 eq) and the corresponding 2-bromoaryl(heteroaryl)aldehyde (0.9 eq) in dry DMF were mixed in a 5 mL microwave vial under N_2 atmosphere. The mixture was stirred at 80 °C under microwave irradiation until starting material disappearance (usually 1 – 2 hours). After cooling to room temperature, NH_4OAc (2.0 eq) was added and the mixture was stirred at 150 °C under microwave irradiation until disappearance of the corresponding Sonogashira product (usually 2 – 3 hours). The mixture was diluted with EtOAc and washed five times with water. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo.* The crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.[56]

3-{1,4-dioxaspiro[4.5]decan-2-yl}isoquinoline (3a): brown oil, 55%, R_f = 0.15 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 2.96 min., m/z = 270.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.95 (d, J = 8.1 Hz, 1H), 7.87 – 7.82 (m, 2H), 7.68 (t, J = 7.5 Hz, 1H), 7.57 (t, J = 7.5 Hz, 1H), 5.39 (t, J = 6.7 Hz, 1H), 4.55 (t, J = 7.5 Hz, 1H), 4.01 (t, J = 7.5 Hz, 1H), 1.86 – 1.63 (m, 8H), 1.49 – 1.46 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 152.1, 136.3, 130.5, 127.9, 127.5, 127.0, 126.7, 116.1, 110.8, 77.8, 70.1, 36.1, 35.2, 25.2, 24.0, 23.9 ppm.

3-{1,4-dioxaspiro[4.5]decan-2-yl}-7-methylisoquinoline (3b): orange oil, 43%, R_f = 0.45 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.94 min., m/z = 284.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.85 (d, J = 4.8 Hz, 1H), 7.79 (s, 1H), 7.61 (s, 1H), 7.41 (dd, J = 1.5 Hz, J = 8.4 Hz, 1H), 5.37 (t, J = 7.0 Hz, 1H), 4.54 (dd, J = 6.8 Hz, J = 8.2 Hz, 1H), 4.00 (dd, J = 6.8 Hz, J = 8.2

Hz, 1H), 2.54 (s, 3H), 1.86 – 1.77 (m, 4H), 1.74 – 1.63 (m, 4H), 1.49 – 1.47 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 151.6, 140.9, 136.6, 129.3, 127.3, 126.4, 125.7, 115.6, 110.8, 77.8, 70.2, 36.1, 35.3, 25.2, 24.1, 23.9, 22.1 ppm.

3-{1,4-dioxaspiro[4.5]decan-2-yl}isoquinolin-7-ol (3c): brown oil, 45%, R_f = 0.25 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.29 min., *m*/*z* = 286.2 [M + H] $^+$. ^{1}H NMR (300 MHz, MeOD) δ 8.99 (s, 1H), 8.21 (s, 1H), 7.85 (s, 1H), 7.38 (dd, *J* = 2.4 Hz, *J* = 8.9 Hz, 1H), 7.30 (d, *J* = 2.4 Hz, 1H), 5.29 (t, *J* = 6.7 Hz, 1H), 4.47 (dd, *J* = 6.6 Hz, *J* = 8.1 Hz, 1H), 3.92 (dd, *J* = 7.1 Hz, *J* = 8.1 Hz, 1H), 1.87 – 1.67 (m, 8H), 1.52 – 1.48 (m, 2H), ppm; ^{13}C NMR (100 MHz, MeOD) δ 158.2, 151.2, 149.7, 131.2, 129.6, 125.2, 122.1, 118.1, 112.0, 109.2, 78.9, 71.2, 37.3, 36.3, 26.4, 25.2, 25.0 ppm.

3-{1,4-dioxaspiro[4.5]decan-2-yl}-5-fluoroisoquinoline (3d): brown oil, 73%, R_f = 0.22 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 3.27 min., *m*/*z* = 288.2 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.18 (s, 1H), 8.08 (s, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.48 (dt, *J* = 5.1 Hz, *J* = 7.9 Hz, 1H), 7.36 – 7.30 (m, 1H), 5.37 (t, *J* = 6.6 Hz, 1H), 4.53 (dd, *J* = 6.8 Hz, *J* = 8.2 Hz, 1H), 4.01 (dd, *J* = 6.6 Hz, *J* = 8.2 Hz, 1H), 1.85 – 1.73 (m, 4H), 1.68 – 1.61 (m, 4H), 1.48 – 1.45 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6 (d, *J* = 254.1 Hz), 154.4, 151.5 (d, *J* = 3.0 Hz), 128.8 (d, *J* = 4.9 Hz), 126.9 (d, *J* = 7.5 Hz), 126.6, 123.2 (d, *J* = 4.4 Hz), 114.0 (d, *J* = 19.1 Hz), 111.0, 109.1 (d, *J* = 3.7 Hz), 77.0, 70.0, 36.1, 35.2, 25.1, 24.0 (d, *J* = 8.3 Hz) ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}furo[2,3-c]pyridine (3e): brown oil, 53%, R_f = 0.22 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 2.54 min., *m*/*z* = 260.2 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.81 (s, 1H), 7.76 (d, *J* = 2.0 Hz, 1H), 6.82 (d, *J* = 1.2 Hz, 1H), 5.32 (t, *J* = 6.7 Hz, 1H), 4.49 (t, *J* = 8.1 Hz, 1H), 3.95 (t, *J* = 7.5 Hz, 1H), 1.81 – 1.75 (m, 5H), 1.71 – 1.64 (m, 3H), 1.50 – 1.46 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 151.5, 148.5, 134.8, 132.8, 112.4, 110.8, 106.3, 77.9, 70.4, 36.2, 35.2, 25.2, 24.1, 23.9 ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}thieno[2,3-c]pyridine (3f): brown oil, 66%, R_f = 0.24 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 2.67 min., m/z = 276.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.05 (s, 1H), 7.95 (s, 1H), 7.72 (d, J = 5.3 Hz, 1H), 7.37 (d, J = 5.3 Hz, 1H), 5.35 (t, J = 6.7 Hz, 1H), 4.52 (t, J = 7.5 Hz, 1H), 3.96 (t, J = 7.5 Hz, 1H), 1.81 – 1.75 (m, 4H), 1.71 – 1.64 (m, 4H), 1.49 – 1.45 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1, 145.6, 143.8, 135.2, 132.5, 123.2, 114.0, 110.8, 77.7, 70.3, 36.1, 35.2, 25.2, 24.0 (d, J = 12.1 Hz) ppm.

6-{1,4-dioxaspiro[4.5]decan-2-yl}-2-methyl-[1,3]thiazolo[5,4-c]pyridine (3g): brown oil, 59%, R_f = 0.23 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.80 min., m/z = 291.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.03 (s, 1H), 5.33 (t, J = 6.6 Hz, 1H), 4.52 (t, J = 7.5 Hz, 1H), 3.97 (t, J = 7.5 Hz, 1H), 2.87 (s, 3H), 1.82 – 1.62 (m, 8H), 1.50 – 1.46 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 154.9, 149.2, 144.8, 143.3, 112.5, 111.0, 77.7, 70.2, 36.2, 35.1, 25.2, 24.1, 23.9, 20.2 ppm.

Synthesis of 4a-g: a stirred solution of the protected annulated pyridine **3a-g** in 1,4-dioxane was cooled to 0 °C using an ice bath. A catalytic amount of concentrated HCl was added. The reaction was stirred at room temperature for 1 – 3 hours. Solvent was evaporated under reduced pressure, the crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.

1-(isoquinolin-3-yl)ethane-1,2-diol (4a): white solid, 90%, R_f = 0.41 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.12 min., m/z = 190.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 9.20 (s, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.94 (d, J = 7.6 Hz, 2H), 7.78 (t, J = 7.3 Hz, 1H), 7.66 (t, J = 7.4 Hz, 1H), 4.95 – 4.92 (m, 1H), 3.98 – 3.88 (m, 1H), 3.79 – 3.70 (m, 1H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 155.5, 152.7, 135.1, 132.3, 128.9, 128.6, 127.8, 124.4, 118.9, 76.2, 67.7 ppm

1-(6-methylisoquinolin-3-yl)ethane-1,2-diol (4b): yellowish solid, 54%, R_f = 0.23 (DCM/MeOH 19:1), UHPLC-ESI-MS: R_t = 1.31 min., m/z = 204.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 9.11 (s,

1H), 7.97 (d, J = 8.4 Hz, 1H), 7.86 (s, 1H), 7.71 (s, 1H), 7.51 (dd, J = 1.4 Hz, J = 8.4 Hz, 1H), 4.95 – 4.91 (m, 1H), 3.93 (dd, J = 4.0 Hz, J = 11.3 Hz, 1H), 3.74 (dd, J = 6.8 Hz, J = 11.3 Hz, 1H), 2.56 (s, 3H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 155.4, 152.2, 143.3, 138.4, 130.9, 128.7, 127.9, 126.7, 118.5, 76.1, 67.7, 22.1 ppm.

1-(7-hydroxyisoquinolin-3-yl)ethane-1,2-diol (4c): yellow oil, 67%, R_f = 0.50 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 0.41 min., m/z = 206.0 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 9.14 (s, 1H), 7.97 (dd, J = 9.4 Hz, J = 16.6 Hz, 2H), 7.51 (dd, J = 1.5 Hz, J = 8.7 Hz, 1H), 7.41 (s, 1H), 4.98 – 4.95 (m, 1H), 3.89 (dd, J = 4.5 Hz, J = 11.3 Hz, 1H), 3.79 (dd, J = 6.2 Hz, J = 11.2 Hz, 1H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 164.9, 159.2, 148.5, 133.8, 130.8, 129.9, 127.5, 120.7, 109.8, 74.4, 67.4 ppm.

1-(5-fluoroisoquinolin-3-yl)ethane-1,2-diol (4d): white solid, 42%, R_f = 0.47 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.44 min., m/z = 208.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 9.25 (s, 1H), 8.11 (s, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.52 – 7.46 (m, 1H), 4.97 – 4.94 (m, 1H), 3.97 (dd, J = 3.9 Hz, J = 11.3 Hz, 1H), 3.77 (dd, J = 6.4 Hz, J = 11.3 Hz, 1H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 158.6 (d, J = 252.4 Hz), 156.3 (d, J = 1.5 Hz), 152.3 (d, J = 2.9 Hz), 130.1 (d, J = 4.6 Hz), 128.4 (d, J = 7.7 Hz), 127.8 (d, J = 17.9 Hz), 124.6 (d, J = 4.4 Hz), 115.3 (d, J = 19.3 Hz), 111.0 (d, J = 3.9 Hz),75.9, 67.3 ppm.

1-{furo[3,2-c]pyridin-6-yl}ethane-1,2-diol (4e): yellow oil, 48%, R_f = 0.33 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 0.48 min., m/z = 180.1 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 8.84 (s, 1H), 8.12 (s, 1H), 7.93 (s, 1H), 7.05 (s, 1H), 4.96 – 4.91 (m, 1H), 3.86 (dd, J = 4.2 Hz, J = 11.2 Hz, 1H), 3.73 (dd, J = 6.6 Hz, J = 11.2 Hz, 1H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 154.7, 152.5, 143.5, 139.9, 132.1, 115.4, 107.7, 75.4, 67.8 ppm.

1-{thieno[3,2-c]pyridin-6-yl}ethane-1,2-diol (4f): white solid, 73%, R_f = 0.35 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 0.64 min., m/z = 196.0 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 8.97 (s, 1H), 7.91 – 7.87 (m, 2H), 7.39 (d, J = 5.3 Hz, 1H), 4.82 (s, 1H), 3.80 (dd, J = 3.9 Hz, J = 11.2 Hz, 1H), 3.63 (dd, J = 6.8 Hz, J = 11.2 Hz, 1H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 156.1, 147.7, 144.5, 136.8, 134.9, 124.3, 116.5, 76.1, 67.9 ppm.

1-{2-methyl-[1,3]thiazolo[4,5-c]pyridin-6-yl}ethane-1,2-diol (4g): yellow oil, 70%, R_f = 0.39 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.12 min., m/z = 211.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 9.03 (s, 1H), 8.16 (s, 1H), 4.91 (s, 1H), 3.90 (dd, J = 3.9 Hz, J = 11.3 Hz, 1H), 3.73 (dd, J = 6.4 Hz, J = 11.1 Hz, 1H), 3.70 (s, 3H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 171.6, 157.2, 150.4, 146.7, 143.2, 115.4, 76.0, 67.7, 19.9 ppm.

4.3 General procedures for the synthesis of 2,4,6-trisubstituted pyrimidines (Series B)

Synthesis of 6a-p: to a stirred solution of **1** (1.0 eq) in dry THF were added $PdCl_2(PPh_3)_2$ (9%mol), CuI (3% mol) and the corresponding acyl chloride (1.5 eq). The reaction was stirred at room temperature for 2 minutes under N_2 atmosphere. Et_3N (1.25 eq) was added and the mixture was stirred at room temperature overnight. Solvent was removed under reduced pressure, the crude redissolved in EtOAc and washed three times with water. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield the corresponding ynone. The products were used without being purified.

Synthesis of 7a-f: to a stirred solution of the corresponding ynone (1.0 eq) in THF was added Na_2CO_3 (2.4 eq) and the corresponding amidine hydrochloride (1.2 eq). The mixture was stirred at reflux overnight. Solvent was evaporated under reduced pressure, the crude was redissolved in EtOAc and

washed three times with water. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.[57]

4-{1,4-dioxaspiro[4.5]decan-2-yl}-2-methyl-6-phenylpyrimidine (7a): orange oil, 42%, R_f = 0.44 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 3.48 min., m/z = 311.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.09 – 8.07 (m, 2H), 7.78 (s, 1H), 7.51 – 7.50 (m, 3H), 5.15 (t, J = 6.6 Hz, 1H), 4.52 (t, J = 7.8 Hz, 1H), 4.01 (dd, J = 6.2 Hz, J = 8.3 Hz, 1H), 2.76 (s, 3H), 1.76 – 1.66 (m, 8H), 1.48 – 1.46 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 167.7, 164.8, 137.2, 130.8, 128.9, 127.3, 111.4, 109.7, 77.3, 69.5, 36.0, 35.0, 26.1, 25.1, 24.0, 23.9 ppm.

2-cyclopropyl-4-{1,4-dioxaspiro[4.5]decan-2-yl}-6-phenylpyrimidine (7b): orange oil, 51%, R_f = 0.51 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 3.85 min., m/z = 337.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.09 – 8.07 (m, 2H), 7.71 (s, 1H), 7.50 – 7.48 (m, 3H), 5.13 (t, J = 6.6 Hz, 1H), 4.49 (t, J = 7.8 Hz, 1H), 4.01 (dd, J = 6.7 Hz, J = 7.8 Hz, 1H), 2.29 – 2.26 (m, 1H), 1.80 – 1.63 (m, 8H), 1.47 (s, 2H), 1.21 (d, J = 4.7 Hz, 2H), 1.09 – 1.05 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 169.6, 164.3, 137.3, 130.7, 128.8, 127.2, 111.3, 109.3, 77.2, 69.5, 36.0, 35.0, 25.1, 24.0, 23.9, 18.0, 10.8 (d, J = 5.2 Hz) ppm.

4-cyclopropyl-6-{1,4-dioxaspiro[4.5]decan-2-yl}-2-phenylpyrimidine (7c): brown oil, 54%, R_f = 0.48 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 3.94 min., m/z = 337.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.44 – 8.41 (m, 2H), 7.47 – 7.44 (m, 3H), 7.31 (s, 1H), 5.17 (t, J = 6.9 Hz, 1H), 4.53 (dd, J = 7.1 Hz, J = 8.4 Hz, 1H), 4.07 (dd, J = 6.3 Hz, J = 8.4 Hz, 1H), 2.09 – 2.02 (m, 1H), 1.82 – 1.64 (m, 8H), 1.51– 1.46 (m, 2H), 1.32 – 1.27 (m, 2H), 1.13 – 1.07 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 168.2, 163.4, 137.8, 130.3, 128.3, 128.1, 112.5, 111.2, 77.0, 69.5, 36.0, 35.1, 25.1, 24.0, 23.8, 17.2, 11.1 (d, J = 7.3 Hz) ppm.

4-cyclopropyl-6-{1,4-dioxaspiro[4.5]decan-2-yl}-2-(pyridin-2-yl)pyrimidine (7d): brown oil, 62%, R_f = 0.52 ($CHCl_3$/MeOH 5:1), UHPLC-ESI-MS: R_t = 2.62 min., m/z = 338.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.82 (ddd, J = 0.9 Hz, J = 1.8 Hz, J = 4.8 Hz, 1H), 8.44 (td, J = 1.0 Hz, J = 8.0 Hz, 1H), 7.82 (dt, J = 1.8 Hz, J = 7.8 Hz, 1H), 7.38 – 7.36 (m, 2H), 5.29 (t, J = 6.6 Hz, 1H), 4.55 (dd, J = 7.1 Hz, J = 8.5 Hz, 1H), 3.99 (dd, J = 6.0 Hz, J = 8.5 Hz, 1H), 2.18 – 2.12 (m, 1H), 1.79 – 1.62 (m, 8H), 1.47– 1.44 (m, 2H), 1.26 – 1.22 (m, 2H), 1.18 – 1.11 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 169.4, 162.6, 155.1, 149.9, 136.8, 124.6, 123.9, 113.0, 111.3, 77.1, 69.8, 36.0, 34.8, 25.1, 24.0, 23.8, 17.5, 11.4 (d, J = 2.8 Hz) ppm.

4-{1,4-dioxaspiro[4.5]decan-2-yl}-2-(3-fluorophenyl)-6-(thiophen-2-yl)pyrimidine (7e): orange oil, 57%, R_f = 0.66 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 4.03 min., m/z = 397.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.33 – 8.30 (m, 1H), 8.24 – 8.19 (m, 1H), 7.85 (dd, J = 1.1 Hz, J = 3.7 Hz, 1H), 7.72 (s, 1H) 7.54 (dd, J = 1.0 Hz, J = 5.0 Hz, 1H), 7.49 – 7.42 (m, 1H), 7.21 – 7.15 (m, 2H), 5.21 (t, J = 6.6 Hz, 1H), 4.56 (dd, J = 7.1 Hz, J = 8.5 Hz, 1H), 4.13 (dd, J = 6.0 Hz, J = 8.5 Hz, 1H), 1.81 – 1.67 (m, 8H), 1.51 – 1.49 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 164.7, 162.7 (d, J = 3.2 Hz), 161.5, 159.6, 142.8, 139.7 (d, J = 7.8 Hz), 130.2, 129.9 (d, J = 8.0 Hz), 128.3, 127.6, 123.9 (d, J = 2.7 Hz), 117.6 (d, J = 21.4 Hz), 115.1 (d, J = 23.2 Hz), 111.4, 109.0, 77.2, 69.4, 36.0, 35.1, 25.1, 24.0, 23.9 ppm.

4-{1,4-dioxaspiro[4.5]decan-2-yl}-2-propyl-6-(thiophen-2-yl)pyrimidine (7f): orange oil, 57%, R_f = 0.57 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 3.77 min., m/z = 345.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (dd, J = 1.0 Hz, J = 3.7 Hz, 1H), 7.61 (s, 1H), 7.48 (dd, J = 1.0 Hz, J = 5.0 Hz, 1H), 7.12 (dd, J = 3.8 Hz, J = 5.0 Hz, 1H), 5.10 (t, J = 6.5 Hz, 1H), 4.48 (dd, J = 7.2 Hz, J = 8.4 Hz, 1H), 3.99 (dd, J = 6.0 Hz, J = 8.4 Hz, 1H), 2.88 (t, J = 7.5 Hz, 2H), 1.89 – 1.80 (m, 2H), 1.74 – 1.62 (m, 8H),

1.45 (s, 2H), 1.00 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.7, 159.3, 143.0, 129.7, 128.2, 127.2, 111.3, 107.8, 76.9, 69.4, 41.1, 35.9, 34.9, 25.1, 24.0, 23.8, 21.8, 13.8 ppm.

Synthesis of 8a-f: a stirred solution of the protected pyrimidine **7a-f** in 1,4-dioxane was cooled to 0 °C using an ice bath. A catalytic amount of concentrated HCl was added. The reaction was stirred at room temperature for 1 – 3 hours. Solvent was evaporated under reduced pressure, the crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.

1-(2-methyl-6-phenylpyrimidin-4-yl)ethane-1,2-diol (8a): yellowish solid, 54%, R_f = 0.58 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.92 min., m/z = 231.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 8.13 – 8.11 (m, 2H), 7.90 (s, 1H), 7.54 – 7.52 (m, 3H), 4.75 – 4.72 (m, 1H), 3.92 (dd, J = 3.8 Hz, J = 11.3 Hz, 1H), 3.78 (dd, J = 5.8 Hz, J = 11.3 Hz, 1H), 2.73 (s, 3H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 172.7, 168.7, 166.2, 138.4, 132.1, 130.1, 128.5, 112.4, 75.7, 67.2, 25.8 ppm.

1-(2-cyclopropyl-6-phenylpyrimidin-4-yl)ethane-1,2-diol (8b): brown oil, 53%, R_f = 0.58 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 2.38 min., m/z = 257.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 8.12 – 8.10 (m, 2H), 7.82 (s, 1H), 7.51 – 7.49 (m, 3H), 4.72 – 4.69 (m, 1H), 3.92 (dd, J = 3.8 Hz, J = 11.3 Hz, 1H), 3.76 (dd, J = 6.0 Hz, J = 11.2 Hz, 1H), 2.31 – 2.24 (m, 1H), 1.19 – 1.18 (m, 2H), 1.09 – 1.06 (m, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 172.6, 172.1, 165.7, 138.5, 132.0, 130.0, 128.3, 111.7, 75.7, 67.2, 18.7, 11.0 ppm.

1-(6-cyclopropyl-2-phenylpyrimidin-4-yl)ethane-1,2-diol (8c): brown oil, 46%, R_f = 0.62 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 2.55 min., m/z = 257.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 8.30 – 8.26 (m, 1H), 7.58 – 7.51 (m, 1H), 7.49 – 7.45 (m, 1H), 7.36 – 7.34 (m, 2H), 7.28 (s, 1H), 4.63 (dd, J = 3.9 Hz, J = 6.1 Hz, 1H), 3.87 (dd, J = 3.9 Hz, J = 11.3 Hz, 1H), 3.69 (dd, J = 6.2 Hz, J = 11.3 Hz, 1H), 2.07 – 2.00 (m, 1H), 1.15 – 1.11 (m, 2H), 1.04 – 1.00 (m, 2H) ppm; ^{13}C NMR (100

MHz, MeOD) δ 174.0, 170.7, 164.6, 139.3, 131.5, 130.1, 129.4, 114.8, 75.7, 67.3, 17.9, 11.6 (d, J = 7.0 Hz) ppm.

1-[6-cyclopropyl-2-(pyridin-2-yl)pyrimidin-4-yl]ethane-1,2-diol (8d): yellowish solid, 67%, R_f = 0.62 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.50 min., m/z = 258.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 8.71 (d, J = 4.2 Hz, 1H), 8.50 (d, J = 7.9 Hz, 1H), 7.99 (t, J = 7.0 Hz, 1H), 7.55 – 7.51 (m, 1H), 7.49 – 7.47 (m, 1H), 4.80 – 4.77 (m, 1H), 3.94 (dd, J = 4.2 Hz, J = 11.3 Hz, 1H), 3.83 (dd, J = 5.8 Hz, J = 11.3 Hz, 1H), 2.23 – 2.17 (m, 1H), 1.27 (d, J = 4.1 Hz, 2H), 1.18 – 1.14 (m, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 174.7, 171.2, 163.2, 156.1, 150.2, 139.0, 126.5, 125.1, 116.3, 75.6, 67.2, 18.0, 11.9 (d, J = 5.3 Hz) ppm.

1-[2-(3-fluorophenyl)-6-(thiophen-2-yl)pyrimidin-4-yl]ethane-1,2-diol (8e): orange solid, 71%, R_f = 0.75 ($CHCl_3$/MeOH 5:1), UHPLC-ESI-MS: R_t = 2.80 min., m/z = 317.0 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 8.29 (d, J = 7.8 Hz, 1H), 8.17 – 8.13 (m, 1H), 7.93 (dd, J = 0.9 Hz, J = 3.7 Hz, 1H), 7.84 (s, 1H), 7.64 (dd, J = 0.9 Hz, J = 5.0 Hz, 1H), 7.51 – 7.43 (m, 1H), 7.23 – 7.16 (m, 2H), 4.80 (dd, J = 3.8 Hz, J = 5.8 Hz, 1H), 4.03 (dd, J = 3.8 Hz, J = 11.3 Hz, 1H), 3.87 (dd, J = 6.0 Hz, J = 11.3 Hz, 1H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 172.6, 166.1, 163.7 (d, J = 3.2 Hz), 162.9, 161.0, 144.0, 141.4 (d, J = 7.8 Hz), 131.5, 131.2 (d, J = 8.1 Hz), 129.4 (d, J = 36.2 Hz), 125.1 (d, J = 2.7 Hz), 118.5 (d, J = 21.6 Hz), 115.8 (d, J = 23.5 Hz), 111.3, 75.8, 67.1 ppm.

1-[2-propyl-6-(thiophen-2-yl)pyrimidin-4-yl]ethane-1,2-diol (8f): yellow solid, 82%, R_f = 0.78 ($CHCl_3$/MeOH 5:1), UHPLC-ESI-MS: R_t = 2.33 min., m/z = 265.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 7.90 (d, J = 3.6 Hz, 1H), 7.78 (s, 1H), 7.63 (d, J = 4.9 Hz, 1H), 7.17 (t, J = 4.5 Hz, 1H), 4.72 – 4.69 (m, 1H), 3.91 (dd, J = 3.8 Hz, J = 11.3 Hz, 1H), 3.75 (dd, J = 6.0 Hz, J = 11.3 Hz, 1H), 2.87 (t, J = 7.5 Hz, 2H), 1.90 – 1.82 (m, 2H), 1.01 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 172.1, 171.7, 161.0, 143.9, 131.4, 129.5, 129.1, 110.4, 75.6, 67.2, 41.8, 22.9, 14.2 ppm.

4.4 General procedures for the synthesis of 2,3,4,6-tetrasubstituted pyridines (Series C)

Synthesis of 9a-b: to a stirred solution of the corresponding ynone (0.6 eq) and ethyl acetoacetate (1.0 eq) in EtOH was added NH_4OAc (10.0 eq). The mixture was stirred at reflux overnight. Solvent was removed under reduced pressure, the crude was redissolved in EtOAc and washed three times with $NaHCO_3$ (saturated solution). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.[53]

ethyl 4-{1,4-dioxaspiro[4.5]decan-2-yl}-2-methyl-6-phenylpyridine-3-carboxylate (9a): brown oil, 92%, R_f = 0.53 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 3.80 min., m/z = 382.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (dd, J = 1.5 Hz, J = 8.0 Hz, 2H), 7.87 (s, 1H), 7.51 – 7.43 (m, 3H), 5.22 (t, J = 6.9 Hz, 1H), 4.47 – 4.39 (m, 3H), 3.72 (dd, J = 7.2 Hz, J = 8.4 Hz, 1H), 2.68 (s, 3H), 1.88 – 1.81 (m, 2H), 1.76 – 1.60 (m, 6H), 1.51 – 1.47 (m, 2H), 1.42 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 158.0, 156.0, 149.2, 138.9, 129.4, 128.8, 127.2, 124.7, 114.4, 110.9, 74.7, 70.9, 61.7, 35.8, 35.0, 25.2, 24.0, 23.8, 23.7, 14.2 ppm.

ethyl 6-cyclopropyl-4-{1,4-dioxaspiro[4.5]decan-2-yl}-2-methylpyridine-3-carboxylate (9b): brown oil, 87%, R_f = 0.50 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 3.42 min., m/z = 346.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.21 (s, 1H), 5.13 (t, J = 6.9 Hz, 1H), 4.40 – 4.33 (m, 3H), 3.64 (d, J = 7.8 Hz, 1H), 2.50 (s, 3H), 2.08 – 1.99 (m, 1H), 1.82 – 1.73 (m, 2H), 1.70 – 1.59 (m, 6H), 1.48 – 1.44 (m, 2H), 1.37 (t, J = 7.1 Hz, 3H), 1.01 – 0.97 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 164.2, 155.5, 148.1, 123.0, 114.4, 110.6, 74.5, 70.9, 61.4, 35.8, 34.8, 25.1, 23.9, 23.8, 23.6, 17.5, 14.2, 10.1 ppm.

Synthesis of 10a-b: a stirred solution of **9a-b** in EtOH was cooled to 0 °C using an ice bath. 1 M NaOH (3.0 eq) was added dropwise. The mixture was stirred at reflux overnight. Solvent was evaporated under reduced pressure, the crude was redissolved in DCM and extracted with water. The

aqueous layer was acidified with 1M HCl until pH = 1 and extracted three times with $CHCl_3$/*i*-PrOH (7:3). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.

4-{1,4-dioxaspiro[4.5]decan-2-yl}-2-methyl-6-phenylpyridine-3-carboxylic acid (10a): yellow oil, 80%, R_f = 0.23 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 2.76 min., m/z = 354.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J = 7.3 Hz, 2H), 7.92 (s, 1H), 7.53 – 7.43 (m, 3H), 5.34 (t, J = 6.9 Hz, 1H), 4.46 (t, J = 7.7 Hz, 1H), 3.73 (t, J = 7.6 Hz, 1H), 2.80 (s, 3H), 1.89 – 1.82 (m, 2H), 1.79 – 1.66 (m, 6H), 1.51 – 1.47 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 158.2, 156.5, 150.7, 138.2, 129.7, 128.8, 127.4, 115.2, 111.0, 74.7, 71.0, 35.8, 34.8, 25.2, 24.0, 23.8, 23.7 ppm.

6-cyclopropyl-4-{1,4-dioxaspiro[4.5]decan-2-yl}-2-methylpyridine-3-carboxylic acid (10b): yellow oil, 83%, R_f = 0.18 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.98 min., m/z = 318.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (s, 1H), 5.40 (t, J = 6.7 Hz, 1H), 4.46 (t, J = 7.9 Hz, 1H), 3.68 (t, J = 7.5 Hz, 1H), 2.77 (s, 3H), 2.54 – 2.49 (m, 1H), 1.71 – 1.59 (m, 8H), 1.42 (s, 2H), 1.22 (d, J = 8.1 Hz, 2H), 1.03 – 1.01 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 159.5, 154.1, 151.1, 131.5, 113.9, 110.8, 74.6, 70.6, 35.8, 34.5, 25.0, 23.9, 23.6, 19.4, 14.4, 11.4, 11.3 ppm.

Synthesis of 11a-b: a stirred solution of the protected pyridine **10a-b** in 1,4-dioxane was cooled to 0 °C using an ice bath. A catalytic amount of concentrated HCl was added. The reaction was stirred at room temperature for 1 – 3 hours. Solvent was evaporated under reduced pressure, the crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.

4-(1,2-dihydroxyethyl)-2-methyl-6-phenylpyridine-3-carboxylic acid (11a): white solid, 80%, R_f = 0.78 ($CHCl_3$/MeOH 5:1), UHPLC-ESI-MS: R_t = 2.34 min., m/z = 274.1 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 – 8.03 (m, 2H), 7.67 (s, 1H), 7.50 – 7.48 (m, 3H), 5.51 (t, J = 4.3 Hz, 1H), 4.15

(dd, J = 3.8 Hz, J = 12.3 Hz, 1H), 3.98 (dd, J = 4.9 Hz, J = 12.3 Hz, 1H), 2.92 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 161.0, 159.8, 156.8, 138.1, 130.3, 128.9, 127.7, 118.6, 111.3, 80.3, 63.2, 21.0 ppm.

6-cyclopropyl-4-(1,2-dihydroxyethyl)-2-methylpyridine-3-carboxylic acid (11b): yellow oil, 72%, R_f = 0.46 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.74 min., m/z = 238.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 7.17 (s, 1H), 5.38 (s, 1H), 3.94 (dd, J = 3.2 Hz, J = 12.3 Hz, 1H), 3.80 (dd, J = 3.8 Hz, J = 12.3 Hz, 1H), 2.61 (s, 3H), 2.13 – 2.04 (m, 1H), 0.99 (d, J = 6.9 Hz, 4H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 171.5, 169.4, 159.8, 158.5, 118.9, 113.4, 82.3, 63.0, 20.6, 18.7, 11.7 (d, J = 7.9 Hz) ppm.

4.5 General procedures for the synthesis of 3,5 -disubstituted (Series D) and 1,3,5-trisubstituted pyrazoles (series E)

Synthesis of 12a-g and 13h-o: to a stirred solution of the corresponding ynone (1.0 eq) in EtOH (2 mL) was added the corresponding hydrazine (hydrazine monohydrated or methyl hydrazine) (1.3 eq). The mixture was stirred at room temperature until starting material consumption (monitored by TLC) (normally 2 – 3 hours). Solvent was removed under reduced pressure, the crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.[58]

5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-phenyl-1*H*-pyrazole (12a): yellowish oil, 57%, R_f = 0.19 (CyH/EtOAC 3:1), UHPLC-ESI-MS: R_t = 2.94 min., m/z = 285.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 7.1 Hz, 2H), 7.42 – 7.30 (m, 3H), 6.55 (s, 1H), 5.21 (t, J = 6.6 Hz, 1H), 4.31 (dd, J = 6.4 Hz, J = 8.1 Hz, 1H), 3.98 (t, J = 8.1 Hz, 1H), 1.76 – 1.61 (m, 8H), 1.43 – 1.40 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.6, 147.9, 130.9, 128.9, 128.3, 125.6, 110.7, 100.5, 71.3, 69.5, 36.1, 35.2, 25.1, 24.0, 23.8 ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-(furan-2-yl)-1*H*-pyrazole (12b): brown oil, 49%, R_f = 0.21 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.75 min., *m*/*z* = 275.2 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (d, *J* = 1.3 Hz, 1H), 6.62 (d, *J* = 3.3 Hz, 1H), 6.47 (s, 1H) 6.44 (dd, *J* = 1.8 Hz, *J* = 3.3 Hz, 1H), 5.21 (t, *J* = 6.6 Hz, 1H), 4.31 (dd, *J* = 6.3 Hz, *J* = 8.3 Hz, 1H), 3.96 (dd, *J* = 6.9 Hz, *J* = 8.2 Hz, 1H), 1.73 – 1.57 (m, 8H), 1.45 – 1.38 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 146.5, 142.1, 139.7, 111.4, 110.7, 106.4, 99.7, 71.1, 69.4, 36.1, 35.2, 25.1, 23.9, 23.8 ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-(thiophen-2-yl)-1*H*-pyrazole (12c): yellow oil, 63%, R_f = 0.32 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.88 min., *m*/*z* = 291.2 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 – 7.24 (m, 2H), 7.03 (dd, *J* = 3.6 Hz, *J* = 5.1 Hz, 1H), 6.42 (s, 1H), 5.19 (t, *J* = 6.5 Hz, 1H), 4.30 (dd, *J* = 6.3 Hz, *J* = 8.3 Hz, 1H), 3.95 (dd, *J* = 6.7 Hz, *J* = 8.3 Hz, 1H), 1.73 – 1.59 (m, 8H), 1.44 – 1.38 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.9, 144.4, 134.5, 127.6, 125.0, 124.1, 110.8, 100.4, 70.8, 69.4, 36.1, 35.1, 25.0, 24.0, 23.8 ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-(propan-2-yl)-1*H*-pyrazole (12d): yellow oil, 68%, R_f = 0.25 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.76 min., *m*/*z* = 251.6 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.09 (s, 1H), 5.15 (t, *J* = 7.2 Hz, 1H), 4.27 (dd, *J* = 6.3 Hz, *J* = 8.2 Hz, 1H), 3.92 (t, *J* = 8.1 Hz, 1H), 2.98 (td, *J* = 6.9 Hz, *J* = 13.9 Hz, 1H), 1.74 – 1.59 (m, 8H), 1.43 – 1.40 (m, 2H), 1.27 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.1, 149.4, 110.4, 99.7, 71.8, 69.5, 36.2, 35.3, 26.3, 25.1, 24.0, 23.8, 22.3 ppm.

3-cyclopropyl-5-{1,4-dioxaspiro[4.5]decan-2-yl}-1*H*-pyrazole (12e): yellow oil, 49%, R_f = 0.12 (CyH/EtOAC 3:1), UHPLC-ESI-MS: R_t = 2.66 min., *m*/*z* = 249.2 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.92 (s, 1H), 5.12 (t, *J* = 6.8 Hz, 1H), 4.25 (dd, *J* = 6.3 Hz, *J* = 8.0 Hz, 1H), 3.89 (t, *J* = 7.7 Hz, 1H), 1.90 – 1.81 (m, 1H), 1.68 – 1.60 (m, 8H), 1.43 – 1.39 (m, 2H), 0.96 – 0.89 (m, 2H), 0.72 –

0.67 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.8, 149.1, 110.3, 99.2, 71.8, 69.5, 36.1, 35.2, 25.1, 23.9, 23.8, 7.6 (d, J = 3.5 Hz), 7.3 ppm.

3-cyclopentyl-5-{1,4-dioxaspiro[4.5]decan-2-yl}-1*H*-pyrazole (12f): yellow oil, 75%, R_f = 0.14 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.01 min., m/z = 277.4 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.08 (s, 1H), 5.14 (t, J = 7.2 Hz, 1H), 4.27 (dd, J = 6.3 Hz, J = 8.1 Hz, 1H), 3.93 (t, J = 7.8 Hz, 1H), 3.07 –3.02 (m, 1H), 2.09 – 2.03 (m, 2H), 1.78 – 1.59 (m 14H), 1.44 – 1.40 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.2, 149.8, 110.3, 100.1, 72.0, 69.5, 37.2, 36.2, 35.3, 33.0, 25.1, 24.0, 23.8 ppm.

3-(adamantan-1-yl)-5-{1,4-dioxaspiro[4.5]decan-2-yl}-1*H*-pyrazole (12g): brown oil, 80%, R_f = 0.28 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.51 min., m/z = 343.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.08 (s, 1H), 5.14 (dd, J = 6.4 Hz, J = 7.5 Hz, 1H), 4.27 (dd, J = 6.3 Hz, J = 8.1 Hz, 1H), 3.93 (t, J = 7.9 Hz, 1H), 2.06 (s, 3H), 1.91 (d, J = 2.6 Hz, 6H), 1.75 – 1.62 (m 14H), 1.44 – 1.40 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 149.5, 110.3, 98.7, 72.1, 69.5, 42.4, 36.5, 36.2, 35.3, 28.3, 25.1, 24.0, 23.8 ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-3-phenyl-1*H*-pyrazole (13a): yellowish oil, 50%, R_f = 0.39 (CyH/EtOAC 3:1), UHPLC-ESI-MS: R_t = 3.22 min., m/z = 299.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J = 7.2 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 7.26 (t, J = 7.2 Hz, 1H), 6.48 (s, 1H), 5.14 (t, J = 6.7 Hz, 1H), 4.31 (dd, J = 6.4 Hz, 1H, J = 8.2 Hz, 1H), 3.94 (s, 3H), 1.66 – 1.60 (m, 8H), 1.44 – 1.40 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.2, 141.5, 133.3, 128.5, 127.6, 125.4, 110.0, 101.6, 69.2, 68.3, 37.2, 36.1, 35.2, 25.0, 23.9 (d, J = 2.4 Hz) ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-(furan-2-yl)-1-methyl-1*H*-pyrazole (13b): brown oil, 51%, R_f = 0.28 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.95 min., m/z = 289.2 $[M + H]^+$. ^{1}H NMR (300

MHz, $CDCl_3$) δ 7.41 (d, *J* = 1.1 Hz, 1H), 6.60 (d, *J* = 3.0 Hz, 1H), 6.43 (t, *J* = 1.8 Hz, 1H), 6.42 (s, 1H) 5.13 (t, *J* = 6.6 Hz, 1H), 4.31 (dd, *J* = 6.3 Hz, *J* = 8.3 Hz, 1H), 4.05 (dd, *J* = 6.9 Hz, *J* = 8.3 Hz, 1H), 3.93 (s, 3H), 1.69 – 1.58 (m, 8H), 1.42 – 1.38 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.7, 142.7, 141.6, 141.4, 111.2, 110.0, 105.3, 101.3, 69.0, 68.2, 37.2, 36.0, 35.2, 25.0, 23.9, 23.8 ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-3-(thiophen-2-yl)-1*H*-pyrazole (13c): yellow oil, 58%, R_f = 0.42 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.13 min., *m*/*z* = 305.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (dd, *J* = 1.0 Hz, *J* = 3.5 Hz, 1H), 7.27 (dd, *J* = 1.0 Hz, *J* = 5.1 Hz, 1H), 7.08 (dd, *J* = 3.6 Hz, *J* = 5.1 Hz, 1H), 6.46 (s, 1H), 5.19 (t, *J* = 6.6 Hz, 1H), 4.38 (dd, *J* = 6.3 Hz, *J* = 8.3 Hz, 1H), 4.11 (dd, *J* = 6.9 Hz, *J* = 8.3 Hz, 1H), 1.73 – 1.67 (m, 8H), 1.51 – 1.45 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.5, 141.7, 136.5, 127.3, 124.2, 123.4, 111.0, 101.5, 69.1, 68.2, 37.2, 36.1, 35.2, 25.0, 23. 9 (d, *J* = 2.4 Hz) ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-3-(propan-2-yl)-1*H*-pyrazole (13d): yellow oil, 71%, R_f = 0.55 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.01 min., *m*/*z* = 265.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.00 (s, 1H), 5.09 (t, *J* = 7.2 Hz, 1H), 4.27 (dd, *J* = 6.3 Hz, *J* = 8.2 Hz, 1H), 3.98 (t, *J* = 7.8 Hz, 1H), 3.85 (s, 3H), 2.92 (td, *J* = 6.9 Hz, *J* = 13.9 Hz, 1H), 1.66 – 1.62 (m, 8H), 1.41 – 1.39 (m, 2H), 1.23 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0, 140.1, 110.7, 101.0, 69.3, 68.3, 36.7, 36.1, 35.2, 27.8, 25.1, 23.9 (d, *J* = 4.0 Hz), 22.9 ppm.

3-cyclopropyl-5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1*H*-pyrazole (13e): yellow oil, 69%, R_f = 0.28 (CyH/EtOAC 3:1), UHPLC-ESI-MS: R_t = 2.88 min., *m*/*z* = 263.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.84 (s, 1H), 5.07 (t, *J* = 6.8 Hz, 1H), 4.26 (dd, *J* = 6.3 Hz, *J* = 8.2 Hz, 1H), 3.95 (dd, *J* = 7.4 Hz, *J* = 8.0 Hz, 1H), 3.82 (s, 3H), 1.91 – 1.82 (m, 1H), 1.64 – 1.60 (m, 8H), 1.41 (d, *J* = 4.8 Hz, 2H), 0.90 – 0.84 (m, 2H), 0.69 – 0.64 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 140.5, 110.8, 100.6, 69.2, 68.3, 36.7, 36.1, 35.2, 25.0, 23.9 (d, *J* = 2.8 Hz), 9.0, 7.7 ppm.

3-cyclopentyl-5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1*H*-pyrazole (13f): brown oil, 66%, R_f = 0.36 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.25 min., *m*/*z* = 291.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.99 (s, 1H), 5.08 (t, *J* = 6.6 Hz, 1H), 4.26 (dd, *J* = 6.3 Hz, *J* = 8.2 Hz, 1H), 3.96 (t, *J* = 7.8 Hz, 1H), 3.84 (s, 3H), 3.06 –2.95 (m, 1H), 2.03 – 1.96 (m, 2H), 1.73 – 1.61 (m 14H), 1.43 – 1.38 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 140.1, 110.7, 101.5, 69.3, 68.3, 39.0, 36.7, 36.1, 35.1, 33.4, 25.3, 25.0, 23.9 (d, *J* = 3.9 Hz) ppm.

3-(adamantan-1-yl)-5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1*H*-pyrazole (13g): brown oil, 61%, R_f = 0.55 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.78 min., *m*/*z* = 357.4 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.02 (s, 1H), 5.09 (t, *J* = 7.2 Hz, 1H), 4.27 (dd, *J* = 6.3 Hz, *J* = 8.2 Hz, 1H), 3.97 (t, *J* = 8.1 Hz, 1H), 3.86 (s, 3H), 2.03 – 1.98 (m, 3H), 1.90 (d, *J* = 2.9 Hz, 6H), 1.75 – 1.73 (m, 6H), 1.65 – 1.61 (m, 8H), 1.44 – 1.39 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 139.7, 110.7, 100.2, 69.3, 68.3, 42.7, 36.8, 36.1, 35.1, 33.8, 28.6, 25.0, 23.9 (d, *J* = 4.5 Hz) ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-3-(3-methylphenyl)-1H-pyrazole (13h): brown oil, 54%, R_f = 0.59 (CyH/EtOAC 9:1), UHPLC-ESI-MS: R_t = 3.34 min., *m*/*z* = 313.2 $[M + H]^+$.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.29 – 7.24 (m, 1H), 7.10 (d, *J* = 7.5 Hz, 1H), 6.49 (s, 1H), 5.17 (t, *J* = 6.7 Hz, 1H), 4.34 (dd, *J* = 6.3 Hz, 8.3 Hz, 1H), 4.08 (dd, *J* = 7.1 Hz, 8.2 Hz, 1H), 3.97 (s, 3H), 1.72 – 1.60 (m, 8H), 1.44 – 1.41 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.1, 141.4, 138.0, 133.1, 128.3, 128.2, 125.9, 122.5, 110.8, 101.4, 69.1, 68.1, 37.0, 36.0, 35.1, 24.9, 23.8 (d, *J* = 3.9 Hz), 21.3 ppm.

3-(5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1H-pyrazol-3-yl)benzonitrile (13i): orange oil, 65%, R_f = 0.30 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.14 min., *m*/*z* = 324.2 $[M + H]^+$.^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.97 (dt, *J* = 1.5 Hz, *J* = 8.1 Hz, 1H), 7.54 (dt, *J* = 1.4 Hz, *J* = 7.7 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 1H), 6.52 (s, 1H), 5.16 (t, *J* = 6.6 Hz, 1H), 4.34 (dd, *J* = 6.3 Hz, *J* = 8.3 Hz,

1H), 4.06 (dd, J = 6.9 Hz, J = 8.3 Hz, 1H), 3.96 (s, 3H), 1.70 – 1.59 (m, 8H), 1.45 – 1.39 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.8, 142.2, 134.6, 130.8, 129.4, 129.3, 128.8, 118.8, 112.7, 111.1, 101.7, 69.0, 68.2, 37.4, 36.1, 35.0, 25.0, 23.8 (d, J = 4.6 Hz) ppm.

3-(3-chloro-5-fluorophenyl)-5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1H-pyrazole (13j): yellow oil, 61%, R_f = 0.49 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.60 min., m/z = 351.0 $[M + H]^+$.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.36 (d, J = 8.9 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 6.47 (s, 1H), 5.14 (t, J = 6.6 Hz, 1H), 4.33 (t, J = 7.5 Hz, 1H), 4.05 (t, J = 7.6 Hz, 1H), 3.94 (s, 3H), 1.67 – 1.65 (m, 8H), 1.42 (d, J = 4.5 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9 (d, J = 248.2 Hz), 147.7 (d, J = 2.9 Hz), 142.1, 136.5 (d, J = 9.3 Hz), 135.1 (d, J = 11.0 Hz), 121.3 (d, J = 3.1 Hz), 114.9 (d, J = 25.0 Hz), 111.1, 110.6 (d, J = 22.8 Hz), 101.8, 69.0, 68.2, 37.3, 36.1, 35.1, 25.0, 23.8 (d, J = 4.0 Hz) ppm.

4-(5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1H-pyrazol-3-yl)benzamide (13k): brown oil, 41%, R_f = 0.29 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.79 min., m/z = 342.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, J = 8.9 Hz, 2H), 6.74 (d, J = 8.9 Hz, 2H), 6.40 (s, 1H), 5.13 (t, J = 6.7 Hz, 1H), 4.31 (dd, J = 6.3 Hz, J = 8.2 Hz, 1H), 4.06 (dd, J = 7.2 Hz, J = 8.2 Hz, 1H), 3.92 (s, 3H), 2.96 (s, 6H), 1.67 – 1.59 (m, 8H), 1.45 – 1.40 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.5, 150.0, 141.2, 126.3, 121.8, 112.5, 110.8, 100.6, 69.1, 68.2, 40.5, 36.9, 36.0, 35.1, 25.0, 23.8 ppm.

5-(5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1H-pyrazol-3-yl)-1,2-oxazole (13l): brown oil, 55%, R_f = 0.17 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.74 min., m/z = 290.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 6.64 (s, 1H), 6.54 (s, 1H), 5.17 (t, J = 6.5 Hz, 1H), 4.35 (dd, J = 6.9 Hz, J = 7.8 Hz, 1H), 4.09 (d, J = 4.6 Hz, 1H), 3.99 (s, 3H), 1.73 – 1.61 (m, 8H), 1.42 (d, J = 4.4 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 150.5, 142.2, 139.4, 111.3, 103.5, 98.6, 68.9, 68.1, 37.7, 36.0, 35.1, 25.0, 23.9 (d, J = 2.2 Hz) ppm.

2-(5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1H-pyrazol-3-yl)-1H-indole (13m): brown oil, 75%, R_f = 0.77 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 3.19 min., m/z = 338.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 9.43 (s br, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.17 (td, J = 6.9 Hz, J = 15.0 Hz, 1H), 7.10 (td, J = 7.2 Hz, J = 14.7 Hz, 1H), 6.74 (d, J = 1.4 Hz, 1H), 6.56 (s, 1H), 5.16 (t, J = 6.6 Hz, 1H), 4.35 (dd, J = 6.4 Hz, J = 8.3 Hz, 1H), 4.10 (dd, J = 6.9 Hz, J = 8.3 Hz, 1H), 3.93 (s, 3H), 1.71 – 1.65 (m, 8H), 1.49 – 1.45 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.8, 141.9, 136.2, 131.6, 128.8, 122.0, 120.4, 119.7, 111.1, 110.8, 101.9, 99.4, 69.0, 68.1, 37.1, 36.1, 35.1, 25.0, 23.9 (d, J = 3.4 Hz) ppm.

3-(5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1H-pyrazol-3-yl)pyridine (13n): brown oil, 46%, R_f = 0.76 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 2.11 min., m/z = 300.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.52 (d, J = 3.8 Hz, 1H), 8.09 (dt, J = 1.9 Hz, 7.9 Hz, 1H), 7.33 (dd, J = 4.9 Hz, 7.8 Hz, 1H), 6.55 (s, 1H), 5.18 (t, J = 6.6 Hz, 1H), 4.36 (dd, J = 6.4 Hz, 8.3 Hz, 1H), 4.08 (dd, J = 7.0 Hz, 8.3 Hz, 1H), 3.98 (s, 3H), 1.72 – 1.60 (m, 8H), 1.46 – 1.41 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 147.0, 146.8, 142.0, 132.7, 129.3, 123.5, 111.1, 101.7, 69.1, 68.2, 37.3, 36.1, 35.1, 25.0, 23.9 (d, J = 4.1 Hz) ppm.

3-cyclohexyl-5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-methyl-1H-pyrazole (13o): brown oil, 55%, R_f = 0.29 (CyH/EtOAC 9:1), UHPLC-ESI-MS: R_t = 3.40 min., m/z = 305.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 5.99 (s, 1H), 5.10 (t, J = 6.9 Hz, 1H), 4.27 (t, J = 8.1 Hz, 1H), 3.98 (t, J = 7.8 Hz, 1H), 3.85 (s, 3H), 2.61 – 2.54 (m, 1H), 1.94 (d, J = 7.8 Hz, 2H), 1.78 (d, J = 4.5 Hz, 2H), 1.66 – 1.62 (m, 8H), 1.43 – 1.33 (m, 6H), 1.29 – 1.20 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.1, 139.9, 110.7, 101.2, 69.2, 68.2, 37.5, 36.7, 36.0, 35.1, 33.3, 26.4, 26.1, 25.0, 23.8 (d, J = 3.8 Hz) ppm.

Synthesis of 14a-g and 15a-g: a stirred solution of the protected pyrazole **12a-g and 13a-g** in 1,4-dioxane was cooled to 0 °C using an ice bath. A catalytic amount of concentrated HCl was added. The

reaction was stirred at room temperature for 1 – 3 hours. Solvent was evaporated under reduced pressure, the crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.

1-(3-phenyl-1*H*-pyrazol-5-yl)ethane-1,2-diol (14a): yellowish solid, 88%, R_f = 0.27 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.76 min., *m*/*z* = 205.4 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 7.74 – 7.71 (m, 2H), 7.43 – 7.38 (m, 2H), 7.34 – 7.29 (m, 1H), 6.62 (s, 1H), 4.81 (dd, *J* = 5.2 Hz, *J* = 6.6 Hz, 1H), 3.82 – 3.72 (m, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 151.6, 149.2, 133.0, 129.8, 129.1, 126.6, 101.2, 69.7, 67.3 ppm.

1-[3-(furan-2-yl)-1*H*-pyrazol-5-yl]ethane-1,2-diol (14b): brown oil, 82%, R_f = 0.29 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.50 min., *m*/*z* = 195.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 7.53 (s, 1H), 6.69 (d, *J* = 3.3 Hz, 1H), 6.50 (d, *J* = 3.6 Hz, 2H), 4.79 (t, *J* = 6.3 Hz, 1H), 3.75 (dd, *J* = 2.5 Hz, *J* = 5.6 Hz, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 150.3, 148.8, 143.4, 141.9, 112.4, 107.1, 100.7, 69.4, 67.2 ppm.

1-[3-(thiophen-2-yl)-1*H*-pyrazol-5-yl]ethane-1,2-diol (14c): yellow oil, 83%, R_f = 0.27 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.68 min., *m*/*z* = 211.0 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 7.35 (dd, *J* = 1.2 Hz, *J* = 4.3 Hz, 2H), 7.06 (dt, *J* = 1.3 Hz, *J* = 4.4 Hz, 1H), 6.49 (s, 1H), 4.80 (t, *J* = 5.9 Hz, 1H), 3.76 – 3.74 (m, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 145.7, 145.5, 136.4, 128.6, 125.8, 125.1, 101.3, 69.2, 67.2 ppm.

1-[3-(propan-2-yl)-1*H*-pyrazol-5-yl]ethane-1,2-diol (14d): brown oil, 87%, R_f = 0.25 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.37 min., *m*/*z* = 171.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 6.12 (s, 1H), 4.72 (t, *J* = 5.9 Hz, 1H), 3.69 (dd, *J* = 2.8 Hz, *J* = 4.1 Hz, 2H), 2.98 (td, *J* = 6.9 Hz, *J* = 13.8 Hz, 1H), 1.27 (dd, *J* = 1.3 Hz, *J* = 6.9 Hz, 6H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 155.1, 151.9, 100.3, 70.0, 67.3, 27.6, 22.9 ppm.

1-(3-cyclopropyl-1*H*-pyrazol-5-yl)ethane-1,2-diol (14e): yellow oil, 89%, R_f = 0.16 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.22 min., *m/z* = 169.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 6.25 (s, 1H), 4.73 (t, *J* = 5.3 Hz, 1H), 3.62 (d, *J* = 5.3 Hz, 2H), 2.00 – 1.91 (m, 1H), 1.14 – 1.07 (m, 2H), 0.87–0.80 (m, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 152.0, 151.7, 99.9, 69.8, 67.3, 8.4, 8.3 ppm.

1-(3-cyclopentyl-1*H*-pyrazol-5-yl)ethane-1,2-diol (14f): brownish solid, 82%, R_f = 0.23 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.68 min., *m/z* = 197.2 $[M + H]^+$. ^1H NMR (300 MHz, CD_3CN) δ 6.01 (s, 1H), 4.63 (t, *J* = 6.9 Hz, 1H), 3.67 – 3.56 (m, 2H), 3.07 – 3.01 (m, 1H), 2.05 – 1.99 (m, 2H), 1.77 – 1.71 (m, 2H), 1.66 – 1.54 (m, 4H) ppm; ^{13}C NMR (100 MHz, CD_3CN) δ 162.4, 152.0, 100.4, 69.06, 67.0, 38.1, 33.8, 25.8 ppm.

1-[3-(adamantan-1-yl)-1*H*-pyrazol-5-yl]ethane-1,2-diol (14g): brownish solid, 75%, R_f = 0.25 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 2.24 min., *m/z* = 263.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 6.08 (s, 1H), 4.71 (dd, *J* = 5.0 Hz, *J* = 7.0 Hz, 1H), 3.70 (dd, *J* = 2.7 Hz, *J* = 6.0 Hz, 2H), 2.04 (s, 3H), 1.95 (d, *J* = 2.7 Hz, 6H), 1.81 (s, 6H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 157.5. 152.0, 99.2, 70.3, 67.4, 43.5, 37.7, 34.3, 30.0 ppm.

1-(1-methyl-3-phenyl-1*H*-pyrazol-5-yl)ethane-1,2-diol (15a): yellowish solid, 79%, R_f = 0.41 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.88, *m/z* = 219.1 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 7.74 (d, *J* = 7.3 Hz, 2H), 7.37 (t, *J* = 7.2 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 1H), 6.62 (s, 1H), 4.82 (t, *J* = 5.8 Hz, 1H), 3.94 (s, 3H), 3.82 (s, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 151.6, 146.4, 134.6, 129.7, 128.8, 126.6, 102.5, 67.6, 66.4, 37.2 ppm.

1-[3-(furan-2-yl)-1-methyl-1*H*-pyrazol-5-yl]ethane-1,2-diol (15b): brown oil, 85%, R_f = 0.42 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.57 min., *m/z* = 209.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 7.50 (d, *J* = 1.7 Hz, 1H), 6.65 (d, *J* = 3.3 Hz, 1H), 6.51 (s, 1H), 6.48 (dd, *J* = 1.8 Hz, *J* = 3.3

Hz, 1H), 4.80 (t, *J* = 6.1 Hz, 1H), 3.92 (s, 3H), 3.80 (dd, *J* = 4.1 Hz, *J* = 6.1 Hz, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 150.0, 146.2, 143.9, 143.1, 112.3, 106.6, 102.2, 67.5, 66.3, 37.2 ppm.

1-[1-methyl-3-(thiophen-2-yl)-1*H*-pyrazol-5-yl]ethane-1,2-diol (15c): yellow oil, 76%, R_f = 0.44 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.76 min., *m*/*z* = 225.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 7.33 – 7.30 (m, 2H), 7.04 (dd, *J* = 3.6 Hz, *J* = 5.1 Hz, 1H), 6.51 (s, 1H), 4.80 (t, *J* = 6.1 Hz, 1H), 3.90 (s, 3H), 3.81 (dd, *J* = 3.8 Hz, *J* = 6.1 Hz, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 146.7, 146.4, 137.4, 128.5, 125.4, 124.8, 102.4, 67.5, 66.3, 37.1 ppm.

1-[1-methyl-3-(propan-2-yl)-1*H*-pyrazol-5-yl]ethane-1,2-diol (15d): brown oil, 77%, R_f = 0.47 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.50 min., *m*/*z* = 185.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 6.09 (s, 1H), 4.74 (t, *J* = 6.0 Hz, 1H), 3.83 (s, 3H), 3.75 (dd, *J* = 4.4 Hz, *J* = 6.2 Hz, 2H), 2.98 (td, *J* = 6.9 Hz, *J* = 13.9 Hz, 1H), 1.22 (d, *J* = 7.0 Hz, 6H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 159.1, 145.3, 101.6, 67.5, 66.3, 36.6, 28.9, 23.3 ppm.

1-(3-cyclopropyl-1-methyl-1*H*-pyrazol-5-yl)ethane-1,2-diol (15e): yellow oil, 92%, R_f = 0.36 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.37 min., *m*/*z* = 183.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 6.40 (s, 1H), 4.87 (t, *J* = 5.8 Hz, 1H), 4.05 (s, 3H), 3.83 (dd, *J* = 5.4 Hz, *J* = 11.3 Hz, 1H), 3.74 (dd, *J* = 6.1 Hz, *J* = 11.2 Hz, 1H), 2.10 – 2.01 (m, 1H), 1.25 – 1.18 (m, 2H), 0.97– 0.91 (m, 2H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 155.0, 146.4, 101.4, 67.5, 66.3, 36.7, 9.5, 8.4 ppm.

1-(3-cyclopentyl-1-methyl-1*H*-pyrazol-5-yl)ethane-1,2-diol (15f): brownish solid, 78%, R_f = 0.28 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 1.79, *m*/*z* = 211.2 $[M + H]^+$. ^{1}H NMR (300 MHz, MeOD) δ 6.08 (s, 1H), 4.74 (t, *J* = 6.2 Hz, 1H), 3.82 (s, 3H), 3.75 (dd, *J* = 4.4 Hz, *J* = 6.2 Hz, 2H), 3.06 – 2.97 (m, 1H), 2.03 – 1.96 (m, 2H), 1.79 – 1.75 (m, 2H), 1.72 – 1.60 (m, 4H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 157.3, 145.3, 102.1, 67.5, 66.3, 40.2, 36.6, 34.5, 26.2 ppm.

1-[3-(adamantan-1-yl)-1-methyl-1*H*-pyrazol-5-yl]ethane-1,2-diol (15g): brownish solid, 67%, R_f = 0.50 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 2.37 min., m/z = 277.2 $[M + H]^+$. ^1H NMR (300 MHz, MeOD) δ 6.10 (s, 1H), 4.74 (t, J = 6.2 Hz, 1H), 3.83 (s, 3H) 3.75 (dd, J = 4.3 Hz, J = 6.1 Hz, 2H), 2.03 (s, 3H), 1.93 (d, J = 2.7 Hz, 6H), 1.80 (s, 6H) ppm; ^{13}C NMR (100 MHz, MeOD) δ 162.2, 144.8, 100.9, 67.6, 66.4, 43.9, 38.0, 36.5, 35.0, 30.2 ppm.

Synthesis of 16a-f: to a stirred solution of **6a** (1.0 eq) in EtOH (2 mL) was added the corresponding hydrazine (1.3 eq). The mixture was stirred at room temperature until starting material consumption (monitored by TLC) (normally 2 – 3 hours). Solvent was removed under reduced pressure, the crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.[58]

5-{1,4-dioxaspiro[4.5]decan-2-yl}-1-ethyl-3-phenyl-1H-pyrazole (16a): orange oil, 44%, R_f = 0.32 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.36 min., m/z = 313.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.79 – 7.76 (m, 2H), 7.40 – 7.35 (m, 2H), 7.31 – 7.28 (m, 1H), 6.49 (s, 1H), 5.17 (t, J = 6.7 Hz, 1H), 4.34 (dd, J = 6.3 Hz, J = 8.2 Hz, 1H), 4.27 (dd, J = 3.6 Hz, J = 7.2 Hz, 2H), 4.09 (dd, J = 7.2 Hz, J = 8.1 Hz, 1H), 1.70 – 1.62 (m, 8H), 1.51 (t, J = 7.2 Hz, 3H), 1.46 – 1.38 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.3, 140.8, 133.5, 128.5, 127.5, 125.5, 110.9, 101.3, 69.0, 68.5, 45.0, 36.1, 35.2, 25.1, 23.9, 16.0 ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-phenyl-1-(propan-2-yl)-1H-pyrazole (16b): brown oil, 60%, R_f = 0.78 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.60 min., m/z = 327.2 $[M + H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, J = 7.2 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.29 – 7.26 (m, 1H), 6.47 (s, 1H), 5.20 (t, J = 7.5 Hz, 1H), 4.69 – 4.61 (m, 1H), 4.34 (dd, J = 6.4 Hz, J = 8.1 Hz, 1H), 4.10 (t, J = 7.5 Hz, 1H), 1.69 – 1.63 (m, 8H), 1.56 (dd, J = 6.7 Hz, J = 8.7 Hz, 6H), 1.46 – 1.42 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.9, 140.2, 133.8, 128.5, 127.3, 125.5, 110.8, 100.8, 69.0, 68.4, 50.9, 36.2, 35.3, 25.1, 23.9, 22.8 (d, J = 1.8 Hz) ppm.

1-cyclopropyl-5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-phenyl-1H-pyrazole (16c): yellow oil, 54%, R_f = 0.74 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.82 min., m/z = 325.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, J = 1.6 Hz, J = 8.0 Hz, 2H), 7.47 – 7.39 (m, 3H), 6.89 (s, 1H), 5.66 (t, J = 5.7 Hz, 1H), 4.51 (dd, J = 7.0 Hz, J = 8.6 Hz, 1H), 3.82 (dd, J = 5.5 Hz, J = 8.6 Hz, 1H), 3.35 – 3.30 (m, 1H), 1.84 – 1.79 (m, 2H), 1.74 – 1.63 (m, 6H), 1.50 – 1.45 (m, 2H), 1.27 – 1.24 (m, 2H), 1.18 – 1.14 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.0, 149.0, 131.9, 129.1, 128.7, 126.3, 110.7, 106.4, 72.5, 69.7, 36.1, 34.5, 25.2, 24.1, 23.8, 11.5 (d, J = 3.1 Hz) ppm.

3-(5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-phenyl-1H-pyrazol-1-yl)propanenitrile (16d): orange oil, 41%, R_f = 0.28 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.15, m/z = 338.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.33 (d, J = 7.2 Hz, 1H), 6.48 (s, 1H), 5.22 (t, J = 6.6 Hz, 1H), 4.65 – 4.48 (m, 2H), 4.39 (t, J = 7.5 Hz, 1H), 4.16 (t, J = 7.6 Hz, 1H), 3.04 (dd, J = 7.2 Hz, J = 14.3 Hz, 2H), 1.69 – 1.55 (m, 8H), 1.44 (d, J = 4.8 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.5, 141.8, 132.7, 128.7, 128.0, 125.6, 117.1, 111.4, 102.0, 68.8, 68.2, 45.5, 36.1, 35.0, 25.0, 24.0, 23.9, 19.1 ppm.

1-cyclopentyl-5-{1,4-dioxaspiro[4.5]decan-2-yl}-3-phenyl-1H-pyrazole (16e): yellow oil, 70%, R_f = 0.59 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.84 min., m/z = 353.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 – 7.78 (m, 2H), 7.37 (t, J = 7.5 Hz, 2H), 7.29 – 7.24 (m, 1H), 6.48 (s, 1H), 5.22 (t, J = 6.7 Hz, 1H), 4.82 – 4.72 (m, 1H), 4.34 (dd, J = 6.3 Hz, J = 8.2 Hz, 1H), 4.09 (dd, J = 7.3 Hz, J = 8.0 Hz, 1H), 2.24 – 2.11 (m, 3H), 2.09 – 1.98 (m, 3H), 1.72 – 1.61 (m, 10H), 1.46 – 1.43 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 141.0, 133.8, 128.4, 127.3, 125.5, 110.8, 100.9, 69.2, 68.5, 60.0, 36.2, 35.3, 33.1 (d, J = 5.8 Hz), 25.1, 24.7 (d, J = 6.2 Hz), 23.9 (d, J = 0.9 Hz) 8 ppm.

5-{1,4-dioxaspiro[4.5]decan-2-yl}-1,3-diphenyl-1H-pyrazole (16f): brown oil, 55%, R_f = 0.81 (CyH/EtOAC 9:1), UHPLC-ESI-MS: R_t = 3.56 min., m/z = 361.2 $[M + H]^+$. ^{1}H NMR (300 MHz,

$CDCl_3$) δ 7.33 – 7.20 (m, 10H), 6.58 (s, 1H), 5.27 (dd, *J* = 6.4 Hz, 7.4 Hz, 1H), 4.38 (dd, *J* = 6.3 Hz, 8.2 Hz, 1H), 4.07 (t, *J* = 7.8 Hz, 1H), 1.79 – 1.59 (m, 8H), 1.48 – 1.41 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.1, 144.1, 139.9, 130.4, 128.8, 128.7, 128.4, 128.2, 127.4, 125.2, 110.4, 105.6, 72.4, 69.6, 36.2, 35.3, 25.1, 24.0, 23.8 ppm.

Synthesis of 18: The compound was synthesized following the general procedure for the synthesis of ynones (using benzoylchloride and the corresponding terminal alkyne) and pyrazoles (using methylhydrazine).

1,5-dimethyl-3-phenyl-1H-pyrazole (18): brownish oil, 90%, R_f = 0.38 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.50 min., *m*/*z* = 173.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.8 Hz, 2H), 7.39 (d, *J* = 7.6 Hz, 2H), 7.30 (d, *J* = 7.3 Hz, 1H), 6.33 (s, 1H), 3.81 (s, 3H), 2.29 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.8, 139.6, 133.6, 128.4. 127.2, 125.3, 102.4, 36.0, 11.1 ppm.

Synthesis of 20a-d: to **1** (1.0 eq) was added the corresponding ketone (3.0 eq) and a catalytic amount of *p*-TSA was added. The reaction was stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude was re-dissolved in Et_2O and washed three times with $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield the corresponding terminal alkynes. The products were used without being purified.

2-ethynyl-1,4-dioxaspiro[4.4]nonane (20a): yellowish oil, 76%, R_f = 0.55 (CyH/EtOAC 9:1), UHPLC-ESI-MS: R_t = 3.02 min., *m*/*z* = 285.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.65 (dt, *J* = 2.1 Hz, *J* = 6.5 Hz, 1H), 4.13 (dd, *J* = 6.6 Hz, *J* = 8.0 Hz, 1H), 3.86 (dd, *J* = 6.4 Hz, *J* = 8.0 Hz, 1H), 2.48 (d, *J* = 2.1 Hz, 1H), 1.98 – 1.89 (m, 1H), 1.86 – 1.84 (m, 1H), 1.81 – 1.75 (m, 2H), 1.73 – 1.64 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 120.2, 81.4, 73.9, 69.8, 64.8, 36.3, 36.1, 23.6, 23.3 ppm.

2-ethynyl-1,4-dioxaspiro[4.6]undecane (20b): yellowish oil, 82%, R_f = 0.48 (CyH/EtOAC 9:1), UHPLC-ESI-MS: R_t = 3.36 min., m/z = 313.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.64 (d, J = 6.0 Hz, 1H), 4.11 (t, J = 7.5 Hz, 1H), 3.87 (t, J = 7.2 Hz, 1H), 2.47 (s, 1H), 1.94 (d, J = 4.5 Hz, 2H), 1.78 (d, J = 7.4 Hz, 2H), 1.55 (s, 8H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 115.2, 81.4, 73.8, 69.3, 64.8, 38.9, 38.7, 29.3, 29.2, 22.4, 22.3 ppm.

2-ethynyl-1,4,8-trioxaspiro[4.5]decane (20c): yellowish oil, 68 %, R_f = 0.33 (CyH/EtOAc 9:1), UHPLC-ESI-MS: R_t = 2.58 min., m/z = 301.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.75 (t, J = 6.1 Hz, 1H), 4.18 (t, J = 6.9 Hz, 1H), 3.98 (t, J = 7.8 Hz, 1H), 3.83 – 3.72 (m, 4H), 2.50 (s, 1H), 1.95 – 1.83 (m, 2H), 1.73 (d, J = 3.5 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 108.2, 81.3, 74.1, 69.6, 65.9 (d, J = 3.7 Hz), 65.1, 36.5, 36.2 ppm.

1-{2-ethynyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl}ethan-1-one (20d): yellowish oil, 72%, R_f = 0.46 (CyH/EtOAC 1:3), UHPLC-ESI-MS: R_t = 2.40 min., m/z = 342.2 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.75 – 4.73 (m, 1H), 4.17 (dd, J = 6.3 Hz, J = 8.1 Hz, 1H), 3.96 (dd, J = 5.9 Hz, J = 8.1 Hz, 1H), 3.70 – 3.63 (m, 2H), 3.54 – 3.48 (m, 2H), 2.50 (d, J = 1.9 Hz, 1H), 2.07 (s, 3H), 1.87 – 1.77 (m, 2H), 1.69 – 1.62 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 108.8, 81.0 (d, J = 13.2 Hz), 74.3 (d, J = 9.1 Hz), 69.7, 65.2, 44.1, 39.3, 35.8 (d, J = 22.0 Hz), 34.9 (d, J = 24.3 Hz), 21.3 ppm.

Synthesis of 22a-d: to a stirred solution of the corresponding ynone (1.0 eq) in EtOH (2 mL) was added methyl hydrazine (1.3 eq). The mixture was stirred at room temperature until starting material consumption (monitored by TLC) (normally 2 – 3 hours). Solvent was removed under reduced pressure, the crude was redissolved in ACN (1 mL), filtered and purified by preparative HPLC.[58]

5-{1,4-dioxaspiro[4.4]nonan-2-yl}-1-methyl-3-phenyl-1H-pyrazole (22a): orange oil, 69%, R_f = 0.38 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.02, m/z = 285.2 $[M + H]^+$. ^{1}H NMR (300 MHz,

$CDCl_3$) δ 7.77 (d, *J* = 7.2, 2H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.30 – 7.25 (m, 1H), 6.51 (s, 1H), 5.09 (t, *J* = 6.7 Hz, 1H), 4.26 (dd, *J* = 6.8 Hz, *J* = 8.1 Hz, 1H), 4.01 (dd, *J* = 7.0 Hz, *J* = 8.0 Hz, 1H), 3.92 (s, 3H), 1.90 – 1.84 (m, 4H), 1.73 – 1.68 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.0, 141.3, 133.2, 128.4, 127.4, 125.3, 120.0, 101.6, 69.1, 68.4, 37.0, 36.4, 36.1, 23.4, 23.3 ppm.

5-{1,4-dioxaspiro[4.6]undecan-2-yl}-1-methyl-3-phenyl-1H-pyrazole (22b): orange oil, 57%, R_f = 0.48 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 3.36, *m*/*z* = 313.2 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 1H), 6.50 (s, 1H), 5.11 (t, *J* = 6.7 Hz, 1H), 4.29 (t, *J* = 7.8 Hz, 1H), 4.01 (t, *J* = 7.7 Hz, 1H), 3.95 (s, 3H), 1.97 – 1.83 (m, 4H), 1.65 – 1.58 (m, 8H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.1, 141.2, 133.2, 128.5, 127.5, 125.4, 114.9, 101.6, 69.1, 68.1, 34.9, 38.3, 37.2, 29.2 (d, *J* = 5.7 Hz), 22.4 (d, *J* = 7.2 Hz) ppm.

1-methyl-3-phenyl-5-{1,4,8-trioxaspiro[4.5]decan-2-yl}-1H-pyrazole (22c): brown oil, 85%, R_f = 0.27 (CyH/EtOAc 3:1), UHPLC-ESI-MS: R_t = 2.58, *m*/*z* = 301.2 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 – 7.75 (m, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.30 (d, *J* = 7.3 Hz, 1H), 6.50 (s, 1H), 5.20 (t, *J* = 6.6 Hz, 1H), 4.37 (dd, *J* = 6.3 Hz, *J* = 8.4 Hz, 1H), 4.14 (dd, *J* = 6.8 Hz, *J* = 8.4 Hz, 1H), 3.96 (s, 3H), 3.82 – 3.75 (m, 4H), 1.85 – 1.78 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.2, 141.1, 133.1, 128.5, 127.6, 125.4, 108.0, 101.4, 69.3, 68.2, 65.9 (d, *J* = 3.7 Hz), 37.2, 36.9, 36.1 ppm.

1-[2-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl]ethan-1-one (22d): yellow oil, 44%, R_f = 0.66 ($CHCl_3$/MeOH 9:1), UHPLC-ESI-MS: R_t = 2.40, *m*/*z* = 342.2 [M + H] $^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.31 (d, *J* = 7.2 Hz, 1H), 6.51 (s, 1H), 5.23 (dd, *J* = 6.1 Hz, *J* = 10.9 Hz, 1H), 4.40 (t, *J* = 6.8 Hz, 1H), 4.17 (dd, *J* = 7.1 Hz, *J* = 14.6 Hz, 1H), 3.97 (s, 3H), 3.74 – 3.68 (m, 2H), 3.59 – 3.51 (m, 2H), 2.12 (d, *J* = 5.6 Hz, 3H), 1.83 – 1.74 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 150.3, 141.1, 132.9, 128.6, 127.8, 125.5,

108.7, 101.4, 69.5 (d, J = 11.7 Hz), 68.4 (d, J = 5.7 Hz), 44.2 (d, J = 4.1 Hz), 39.5, 35.6 (d, J = 3.6 Hz), 21.3 ppm.

4.2 Biology

LsrK overexpression and purification

LsrK from *S. typhimurium* was overexpressed in *E. coli* MET1158 [*E. coli*, amp resistance, BL21 (DE3) luxS-, with pMET1144 (lsrK-His in pET21b)][59], kindly donated by Prof. Karina Xavier (Instituto Gulbenkian de Ciência, Portugal), according to a previously reported protocol.[54]

Primary screening and dose response experiments

Each compound was initially tested against LsrK at 200 µM. Compounds were plated in triplicate and 300 nM LsrK, 300 µM (*S*)-DPD and 100 µM ATP, diluted in assay buffer (25 mM triethanolamine, pH 7.4, 200 µM $MgCl_2$ and 0.1 mg/mL BSA). After 15mintes of incubation, Kinase Glo Luminescence kit´s (Promega, USA) reagent was added according to manufacturer´s instructions. Luminescence was recorded after 30 minutes with Varioskan LUX plate reader (Thermo Fisher Scientific, Finland). Compounds showing an inhibition > 40 % were tested in a dose-response experiment against LsrK at 6 different concentrations (25µM – 500 µM). The assay was carried out as reported above, except for the addition of Triton X-100 to the assay buffer to prevent potential aggregation. IC_{50} values were determined using the four parameters logistic function in Origin 8.6.

Glycerokinase assay

Compounds active against LsrK were also tested against glycerokinase to evaluate their selectivity. 0.3 U/mL glycerokinase from *E. coli* (Sigma-Aldrich, USA) and 300 µM glycerol were added to plate followed by 100 µM ATP. The assay was performed according to the LsrK inhibition assay protocol.

4.3 Molecular Modelling

All the computations were carried out using Schrödinger suite 2018-1.[60] The crystal structure of LsrK (5YA1) was downloaded from RCSB PDB. The protein structure was prepared using protein preparation wizard of Schrödinger so that both ATP and substrate were included in the process. Hydrogens were added to the structure using default settings. Missing loops and side chains (residues 363-372, located far away from the active site) were filled using Prime. Water molecules more than 5Å away from the ATP and substrate atoms were deleted. Hetero atom ionization states were generated, optimized and then minimized. The active site was defined for grid generation for docking based on the substrate coordinates. All synthesized compounds were prepared suing Ligprep module in Schrödinger. Ionization states were generated using Epik. Tautomers and stereoisomers were generated retaining the specified chiralities. Prepared ligands and protein were used as input for docking in Glide module of Schrödinger. Docking was done using standard precision mode in Glide.

ABBREVIATIONS

(2R,4*S*)-2,4-dihydroxy-2-methyldihydrofuran-3-one	*R*-DHMF
(2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran	*R*-THMF
(2*S*,4*S*)-2,4-dihydroxy-2-methyldihydrofuran-3-one	*S*-DHMF
(2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran	*S*-THMF
(2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuranborate	*S*-THMF-borate
2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl	Sphos
3,4,4-trihydroxy-2-pentanone-5-phosphate	P-TPO
4,5-dihydroxy-2,3-pentanedione	DPD
Acetonitrile	ACN
Ammonium acetate	NH_4OAc

Autoinducer-2	AI-2
Autoinducers	AIs
Bis(acetonitrile)dichloropalladium(II)	$PdCl_2(CH_3CN)_2$
Bis(triphenylphosphine)palladium(II) dichloride	$PdCl_2(PPh_3)_2$
Broad	br
Carbon nuclear magnetic resonance	^{13}C NMR
Cesium carbonate	Cs_2CO_3
Copper iodide	CuI
Deuterated chloroform	$CDCl_3$
Deuterated methanol	MeOD
Diethyl ether	Et_2O
Dihydroxyacetone phosphate	DHAP
DPD-inspired heterocycles	DPD-IHs
Doublet	d
Doublet of doublets	dd
Doublet of triplets	dt
Ethanol	EtOH
Hydrochloric acid	HCl
Id est	i.e.
LuxS regulated	Lsr
Magnesium chloride	$MgCl_2$
Magnesium sulphate	$MgSO_4$
Methanol	MeOH
Methylhydrazine	$MeNHNH_2$

Micromolar	µM
Microwave	mW
Milligram	mg
N-acyl homoserine lactones	AHLs
Palladium acetate	$Pd(OAc)_2$
para-toluen sulfonic acid	*p*-TSA
Potassium acetate	KOAc
Protein Data Bank	PDB
Proton nuclear meagnetic resonance	^{1}H NMR
rt	Room temperature
R_t	Retention time
Quorum Sensing	QS
Quorum Sensing inhibitors	QSI
S-3,3,4,5-tetrahydroxy-2-pentanone	*S*-THP
S-3,3,4,5-tetrahydroxy-2-pentanone-5-phosphate	P-DPD
S-4,5-dihydroxy-2,3-pentanedione	*S*-DPD
S-adenosylmethionine	SAM
S-adenosylhomocysteine	SAH
Sodium carbonate decahydrated	Na_2CO_3*$10H_2O$
Sodium hydroxide	NaOH
S-ribosylhomocisteine	SRH
Triethylamine	Et_3N
Triplet of doublet	td
Triphenylphosphine	PPh_3

Corresponding Author

Simona Collina: simona.collina@unipv.it; Tel.: (+39) 0382-987379

Present Address

§ Current address: DE Shaw Research, 120W 45th Street, 10036, New York, NY

Author Contributions

Conceptualization, S.S., F.G and S.C.; Methodology, S.S., F.G.; Investigation, S.S., V.G., M.P., F.G., S.C., P.T., P.M., T.L., A.P.; Resources, A.P., P.T.; Writing-Original Draft Preparation, S.S., V.G., P.T., P.M., A.P.; Writing-Review & Editing, S.S., F.G., P.T., A.P., S.C.; Supervision, S.C.; Project Administration, A.K., P.T., A.P.; Funding Acquisition, F.G., P.T., A.P.

Funding Sources

This research was funded by the European Union's Horizon 2020 research and innovation programme INTEGRATE under the Marie Sklodowska Curie grant agreement N°. 642620.

The authors declare no competing financial interest

ACKNOWLEDGMENT

Martyna Bielska, Xenia Iwanova, Virginia Llemos and Eduard Ackerman are acknowledged for technical assistance.

Supporting Information availability: a supporting information file containing figures and NMRs is available. Molecular Formula Strings as .csv file is also available.

References

(1) Surette, M. G.; Miller, M. B.; Bassler, B. L. Quorum Sensing in Escherichia Coli, Salmonella Typhimurium, and Vibrio Harveyi: A New Family of Genes Responsible for Autoinducer Production. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96* (4), 1639–1644.

(2) Waters, C. M.; Bassler, B. L. Quorum Sensing: Cell-to-Cell Communication in Bacteria. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 319–346.

(3) Bassler, B. L.; Losick, R. Bacterially Speaking. *Cell* **2006**, *125* (2), 237–246.

(4) Lowery, C. A.; Dickerson, T. J.; Janda, K. D. Interspecies and Interkingdom Communication Mediated by Bacterial Quorum Sensing. *Chem. Soc. Rev.* **2008**, *37* (7), 1337–1346.

(5) Ng, W.-L.; Bassler, B. L. Bacterial Quorum-Sensing Network Architectures. *Annu. Rev. Genet.* **2009**, *43* (1), 197–222.

(6) Davies, D. G.; Parsek, M. R.; Pearson, J. P.; Iglewski, B. H.; Costerton, J. W.; Greenberg, E. P. The Involvement of Cell-to-Cell Signals in the Development of a Bacterial Biofilm. *Science* **1998**, *280* (5361), 295–298.

(7) Rickard, A. H.; Palmer, R. J.; Blehert, D. S.; Campagna, S. R.; Semmelhack, M. F.; Egland, P. G.; Bassler, B. L.; Kolenbrander, P. E. Autoinducer 2: A Concentration-Dependent Signal for Mutualistic Bacterial Biofilm Growth. *Mol. Microbiol.* **2006**, *60* (6), 1446–1456.

(8) Irie, Y.; Parsek, M. R. Quorum Sensing and Microbial Biofilms. *Curr. Top. Microbiol. Immunol.* **2008**, *322*, 67–84.

(9) Ahmed, N. A. A. M.; Petersen, F. C.; Scheie, A. A. AI-2 Quorum Sensing Affects Antibiotic Susceptibility in Streptococcus Anginosus. *J. Antimicrob. Chemother.* **2007**, *60* (1), 49–53.

(10) Suga, H.; Smith, K. M. Molecular Mechanisms of Bacterial Quorum Sensing as a New Drug Target. *Curr. Opin. Chem. Biol.* **2003**, *7* (5), 586–591.

(11) Geske, G. D.; Wezeman, R. J.; Siegel, A. P.; Blackwell, H. E. Small Molecule Inhibitors of Bacterial Quorum Sensing and Biofilm Formation. *J. Am. Chem. Soc.* **2005**, *127* (37), 12762–12763

(12) Rasmussen, T. B.; Givskov, M. Quorum-Sensing Inhibitors as Anti-Pathogenic Drugs. *Int. J. Med. Microbiol.* **2006**, *296* (2–3), 149–161.

(13) Clatworthy, A. E.; Pierson, E.; Hung, D. T. Targeting Virulence: A New Paradigm for Antimicrobial Therapy. *Nat. Chem. Biol.* **2007**, *3* (9), 541–548.

(14) Federle, M. J.; Bassler, B. L. Interspecies Communication in Bacteria. *J. Clin. Invest.* **2003**, *112* (9), 1291–1299.

(15) Herzberg, M.; Kaye, I. K.; Peti, W.; Wood, T. K. YdgG (TqsA) Controls Biofilm Formation in Escherichia Coli K-12 through Autoinducer 2 Transport. *J. Bacteriol.* **2006**, *188* (2), 587–598.

(16) Rettner, R. E.; Saier, M. H. The Autoinducer-2 Exporter Superfamily. *J. Mol. Microbiol. Biotechnol.* **2010**, *18* (4), 195–205.

(17) Globisch, D.; Lowery, C. A.; McCague, K. C.; Janda, K. D. Uncharacterized 4,5-Dihydroxy-2,3-Pentanedione (DPD) Molecules Revealed Through NMR Spectroscopy: Implications for a Greater Signaling Diversity in Bacterial Species. *Angew. Chem. Int. Ed.* **2012**, *51* (17), 4204–4208.

(18) Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczer, I.; Bassler, B. L.; Hughson, F. M. Structural Identification of a Bacterial Quorum-Sensing Signal Containing Boron. *Nature* **2002**, *415* (6871), 545–549.

(19) Miller, S. T.; Xavier, K. B.; Campagna, S. R.; Taga, M. E.; Semmelhack, M. F.; Bassler, B. L.; Hughson, F. M. Salmonella Typhimurium Recognizes a Chemically Distinct Form of the Bacterial Quorum-Sensing Signal AI-2. *Mol. Cell* **2004**, *15* (5), 677–687.

(20) Ha, J.-H.; Eo, Y.; Grishaev, A.; Guo, M.; Smith, J. A. I.; Sintim, H. O.; Kim, E.-H.; Cheong, H.-K.; Bentley, W. E.; Ryu, K.-S. Crystal Structures of the LsrR Proteins Complexed with Phospho-AI-2 and Two Signal-Interrupting Analogues Reveal Distinct Mechanisms for Ligand Recognition. *J. Am. Chem. Soc.* **2013**, *135* (41), 15526–15535.

(21) Xavier, K. B.; Bassler, B. L. Regulation of Uptake and Processing of the Quorum-Sensing Autoinducer AI-2 in Escherichia Coli. *J. Bacteriol.* **2005**, *187* (1), 238–248.

(22) Marques, J. C.; Lamosa, P.; Russell, C.; Ventura, R.; Maycock, C.; Semmelhack, M. F.; Miller, S. T.; Xavier, K. B. Processing the Interspecies Quorum-Sensing Signal Autoinducer-2 (AI-2): Characterization of Phospho-(S)-4,5-Dihydroxy-2,3-Pentanedione Isomerization by LsrG Protein. *J. Biol. Chem.* **2011**, *286* (20), 18331–18343.

(23) Penesyan, A.; Gillings, M.; Paulsen, I. T. Antibiotic Discovery: Combatting Bacterial Resistance in Cells and in Biofilm Communities. *Mol. Basel Switz.* **2015**, *20* (4), 5286–5298.

(24) Kociolek, M. Quorum-Sensing Inhibitors and Biofilms. *Anti-Infect. Agents Med. Chem.* **2009**, *8* (4), 315–326.

(25) Brackman, G.; Coenye, T. Quorum Sensing Inhibitors as Anti-Biofilm Agents. *Curr. Pharm. Des.* **2015**, *21* (1), 5–11.

(26) Mandabi, A.; Ganin, H.; Meijler, M. M. Synergistic Activation of Quorum Sensing in Vibrio Harveyi. *Bioorg. Med. Chem. Lett.* **2015**, *25* (18), 3966–3969.

(27) Lowery, C. A.; Park, J.; Kaufmann, G. F.; Janda, K. D. An Unexpected Switch in the Modulation of AI-2-Based Quorum Sensing Discovered through Synthetic 4,5-Dihydroxy-2,3-Pentanedione Analogues. *J. Am. Chem. Soc.* **2008**, *130* (29), 9200–9201.

(28) Ganin, H.; Tang, X.; Meijler, M. M. Inhibition of Pseudomonas Aeruginosa Quorum Sensing by AI-2 Analogs. *Bioorg. Med. Chem. Lett.* **2009**, *19* (14), 3941–3944.

(29) Smith, J. A. I.; Wang, J.; Nguyen-Mau, S.-M.; Lee, V.; Sintim, H. O. Biological Screening of a Diverse Set of AI-2 Analogues in Vibrio Harveyi Suggests That Receptors Which Are Involved in Synergistic Agonism of AI-2 and Analogues Are Promiscuous. *Chem. Commun.* **2009**, No. 45, 7033.

(30) Roy, V.; Smith, J. A. I.; Wang, J.; Stewart, J. E.; Bentley, W. E.; Sintim, H. O. Synthetic Analogs Tailor Native AI-2 Signaling across Bacterial Species. *J. Am. Chem. Soc.* **2010**, *132* (32), 11141–11150.

(31) Gamby, S.; Roy, V.; Guo, M.; Smith, J. A. I.; Wang, J.; Stewart, J. E.; Wang, X.; Bentley, W. E.; Sintim, H. O. Altering the Communication Networks of Multispecies Microbial Systems Using a Diverse Toolbox of AI-2 Analogues. *ACS Chem. Biol.* **2012**, *7* (6), 1023–1030.

(32) Frezza, M.; Balestrino, D.; Soulère, L.; Reverchon, S.; Queneau, Y.; Forestier, C.; Doutheau, A. Synthesis and Biological Evaluation of the Trifluoromethyl Analog of (4S)-4,5-Dihydroxy-2,3-Pentanedione (DPD). *Eur. J. Org. Chem.* **2006**, *2006* (20), 4731–4736.

(33) Kadirvel, M.; Fanimarvasti, F.; Forbes, S.; McBain, A.; Gardiner, J. M.; Brown, G. D.; Freeman, S. Inhibition of Quorum Sensing and Biofilm Formation in Vibrio Harveyi by 4-Fluoro-DPD; a Novel Potent Inhibitor of Signalling. *Chem. Commun. Camb. Engl.* **2014**, *50* (39), 5000–5002.

(34) Zhang, Y.; Zagnitko, O.; Rodionova, I.; Osterman, A.; Godzik, A. The FGGY Carbohydrate Kinase Family: Insights into the Evolution of Functional Specificities. *PLoS Comput Biol* **2011**, *7* (12), e1002318.

(35) Tsuchikama, K.; Zhu, J.; Lowery, C. A.; Kaufmann, G. F.; Janda, K. D. C4-Alkoxy-HPD: A Potent Class of Synthetic Modulators Surpassing Nature in AI-2 Quorum Sensing. *J. Am. Chem. Soc.* **2012**, *134* (33), 13562–13564.

(36) Selvam, T. P.; James, C. R.; Dniandev, P. V.; Valzita, S. K. A Mini Review of Pyrimidine and Fused Pyrimidine Marketed Drugs. *Res. Pharm.* **2012**, *2* (4), 1 – 9.

(37) Yerragunta, V.; Patil, P.; Anusha, V.; KumaraSwamy, T.; Suman, D.; Samhitha, T. Pyrimidine and Its Biological Activity: A Review. *PharmaTutor* **2013**, *1* (2), 39–44.

(38) Kartsev, V. G. Natural Compounds In Drug Discovery. Biological Activity and New Trends in the Chemistry of Isoquinoline Alkaloids. *Med. Chem. Res.* **2004**, *13* (6–7), 325–336.

(39) Galán, A.; Moreno, L.; Párraga, J.; Serrano, Á.; Sanz, M. J.; Cortes, D.; Cabedo, N. Novel Isoquinoline Derivatives as Antimicrobial Agents. *Bioorg. Med. Chem.* **2013**, *21* (11), 3221–3230.

(40) Iranshahy, M.; Quinn, R. J.; Iranshahi, M. Biologically Active Isoquinoline Alkaloids with Drug-like Properties from the Genus Corydalis. *RSC Adv.* **2014**, *4* (31), 15900–15913.

(41) Chlebek, J.; Novák, Z.; Kassemová, D.; Šafratová, M.; Kostelník, J.; Malý, L.; Ločárek, M.; Opletal, L.; Hošťálková, A.; Hrabinová, M.; Kuneš, J.; Novotná, P.; Urbanová, M.; Nováková, L.; Macáková, K.; Hulcová, D.; Solich, P.; Pérez Martín, C.; Jun, D.; Cahlíková, L. Isoquinoline Alkaloids from Fumaria Officinalis L. and Their Biological Activities Related to Alzheimer's Disease. *Chem. Biodivers.* **2016**, *13* (1), 91–99.

(42) Marty, M.; Fumoleau, P.; Adenis, A.; Rousseau, Y.; Merrouche, Y.; Robinet, G.; Senac, J.; Puozzo, C. Oral Vinorelbine Pharmacokinetics and Absolute Bioavailability Study in Patients with Solid Tumors. *Ann. Oncol.* **2001**, *12* (11), 1643–1649.

(43) Faller, B. A.; Pandit, T. N. Safety and Efficacy of Vinorelbine in the Treatment of Non-Small Cell Lung Cancer. *Clin. Med. Insights Oncol.* **2011**, *5*, CMO.S5074.

(44) Chadha, N.; Silakari, O. Indoles as Therapeutics of Interest in Medicinal Chemistry: Bird's Eye View. *Eur. J. Med. Chem.* **2017**, *134*, 159–184. https://doi.org/10.1016/j.ejmech.2017.04.003.

(45) Altaf, A. A.; Shahzad, A.; Gul, Z.; Rasool, N.; Badshah, A.; Lal, B.; Khan, E. A Review on the Medicinal Importance of Pyridine Derivatives. *J. Drug Des. Med. Chem.* **2015**, *1* (1), 1.

(46) Lucas, S. The Pharmacology of Indomethacin. *Headache J. Head Face Pain* **2016**, *56* (2), 436–446.

(47) Naim, M. J.; Alam, O.; Nawaz, F.; Alam, M. J.; Alam, P. Current Status of Pyrazole and Its Biological Activities. *J. Pharm. Bioallied Sci.* **2016**, *8* (1), 2–17. https://doi.org/10.4103/0975-7406.171694.

(48) Faria, J. V.; Vegi, P. F.; Miguita, A. G. C.; dos Santos, M. S.; Boechat, N.; Bernardino, A. M. R. Recently Reported Biological Activities of Pyrazole Compounds. *Bioorg. Med. Chem.* **2017**, *25* (21), 5891–5903.

(49) Stotani, S.; Gatta, V.; Medda, F.; Padmanaban, M.; Karawajczyk, A.; Tammela, P.; Giordanetto, F.; Tzalis, D.; Collina, S. A Versatile Strategy for the Synthesis of 4,5-Dihydroxy-2,3-Pentanedione (DPD) and Related Compounds as Potential Modulators of Bacterial Quorum Sensing. *Molecules* **2018**, *23* (10), 2545.

(50) Karpov, A. S.; Müller, T. J. J. Straightforward Novel One-Pot Enaminone and Pyrimidine Syntheses by Coupling-Addition-Cyclocondensation Sequences. *Synthesis* **2003**, *2003* (18), 2815–2826.

(51) Palimkar, S. S.; Kumar, P. H.; Jogdand, N. R.; Daniel, T.; Lahoti, R. J.; Srinivasan, K. V. Copper-, Ligand- and Solvent-Free Synthesis of Ynones by Coupling Acid Chlorides with Terminal Alkynes. *Tetrahedron Lett.* **2006**, *47* (31), 5527–5530.

(52) Cox, R. J.; Ritson, D. J.; Dane, T. A.; Berge, J.; Charmant, J. P. H.; Kantacha, A. Room Temperature Palladium Catalysed Coupling of Acyl Chlorides with Terminal Alkynes. *Chem. Commun.* **2005**, *8*, 1037–1039.

(53) Xiong, X.; Bagley, M. C.; Chapaneri, K. A New Mild Method for the One-Pot Synthesis of Pyridines. *Tetrahedron Lett.* **2004**, *45* (32), 6121–6124.

(54) Medarametla, P.; Gatta, V.; Kajander, T.; Laitinen, T.; Tammela, P.; Poso, A. Cover Feature: Structure-Based Virtual Screening of LsrK Kinase Inhibitors to Target Quorum Sensing. *ChemMedChem* **2018**, *13* (22), 2350–2350.

(55) Walsh, F. Superbugs to Kill "More than Cancer." *BBC News*. December 11, 2014.

(56) Yang, D.; Burugupalli, S.; Daniel, D.; Chen, Y. Microwave-Assisted One-Pot Synthesis of Isoquinolines, Furopyridines, and Thienopyridines by Palladium-Catalyzed Sequential Coupling–Imination–Annulation of 2-Bromoarylaldehydes with Terminal Acetylenes and Ammonium Acetate. *J. Org. Chem.* **2012**, *77* (9), 4466–4472.

(57) Karpov, A. S.; Müller, T. J. J. Straightforward Novel One-Pot Enaminone and Pyrimidine Syntheses by Coupling-Addition-Cyclocondensation Sequences. *Synthesis* **2003**, *2003* (18), 2815–2826.

(58) Trost, B. M.; Hung, C.-I. (Joey). Broad Spectrum Enolate Equivalent for Catalytic Chemo-, Diastereo-, and Enantioselective Addition to N-Boc Imines. *J. Am. Chem. Soc.* **2015**, *137* (50), 15940–15946.

(59) Xavier, K. B.; Miller, S. T.; Lu, W.; Kim, J. H.; Rabinowitz, J.; Pelczer, I.; Semmelhack, M. F.; Bassler, B. L. Phosphorylation and Processing of the Quorum-Sensing Molecule Autoinducer-2 in Enteric Bacteria. *ACS Chem. Biol.* **2007**, *2* (2), 128–136.

(60) Small-Molecule Drug Discovery Suite | Schrödinger. https://www.schrodinger.com/suites/small-molecule-drug-discovery-suite

Table of Content graphic

