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Antibacterial screening of new bis(pyrazolo[1,5-*a*]pyrimidine) hybrids linked to different spacers

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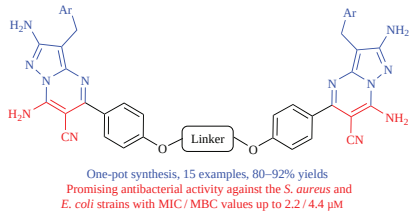
A three-component protocol was adopted to efficiently prepare new bis(pyrazolo[1,5-*a*]pyrimidines) linked to different spacers in 80–92% yields from 1*H*-pyrazole-3,5-diamines, malononitrile and bis(aldehydes). The *p*-xylene-linked bis(pyrimidine) bearing 3-positioned 4-methoxybenzyl substituent had the best antibacterial activity with MIC / MBC values up to 2.2 / 4.4 μM.



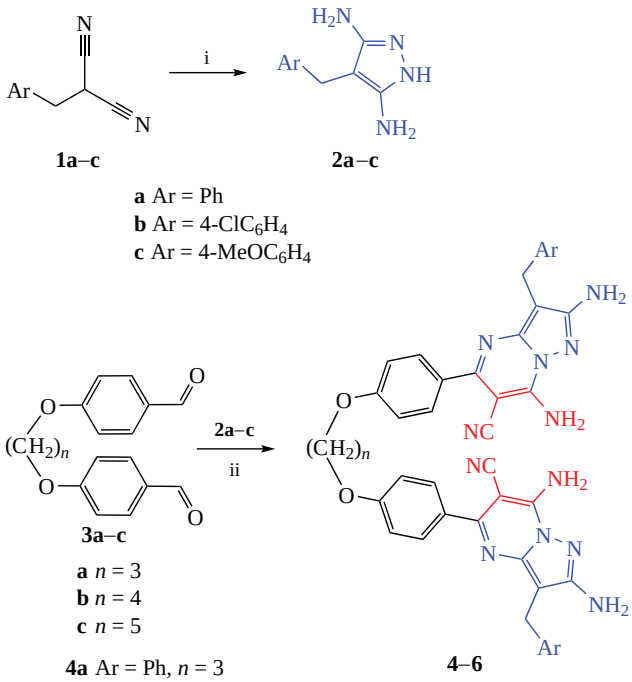
Keywords: bis(aldehyde), pyrazolo[1,5-*a*]pyrimidine, multicomponent reactions, aza-Michael addition, α,β-unsaturated nitriles, heterocyclization, 1,3-dielectrophiles, *in vitro* antibacterial screening.

Antibacterial resistance has attained dangerous levels in all parts of the world over the last two decades. Antibiotic resistance, which was once largely confined to hospitals and long-term care facilities, has now spread to community settings and is one of the most pressing global public health concerns.[1] Antimicrobial resistance has been identified as one of the most serious threats to human health by the World Health Organization (WHO). Much effort has gone into developing new antimicrobial agents and combating antimicrobial resistance.[2]

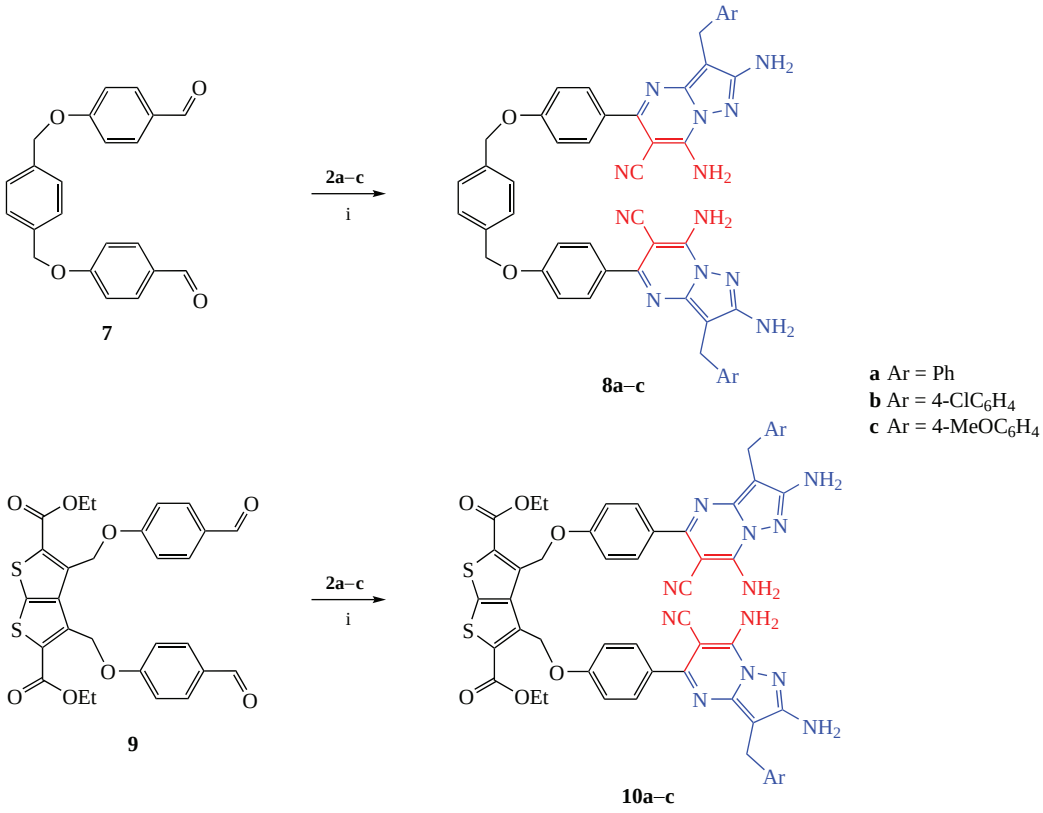
Pyrazolo[1,5-*a*]pyrimidines are promising drug-like purine analogues with a variety of biological applications as antimetabolites in purine biochemical interactions.[3] The previous scaffolds demonstrate excellent antibacterial,[4] antifungal activity,[5] and potential selective inhibitory activity against COX enzymes.[6] Additionally, they have an effect on the central nervous system because they could be used as benzodiazepine receptor ligands,[7] and anxiolytic agents,[8] in addition to their inhibitory activity against KDR kinases.[9] They also showed significant anti-proliferative activity,[10] as well as promising inhibitory activity against various enzymes that are used as effective targets in chemotherapy, including cyclin-dependent[11] and pim kinases.[12]

Synthesis of pyrazolo[1,5-*a*]pyrimidines is usually carried out by reacting 3(5)-aminopyrazoles with various 1,3-dielectrophilic reagents, including amino enones,[13] sodium salts of formyl ketones,[14] α,β-unsaturated nitriles,[15] α,β-unsaturated ketones,[16] 1,3-diketones,[17] and β-keto esters.[18] In the context of our ongoing effort to synthesize new pyrazole-fused azines with promising antibacterial activity,[19] we report herein a one-pot protocol to prepare new bis(pyrazolo[1,5-*a*]-pyrimidines) linked to different spacers *via* *p*-phenoxy units.

At first, the appropriate malononitriles **1a**–**c** were reacted with hydrazine hydrate to afford 1*H*-pyrazole-3,5-diamines **2a**–**c** (Scheme 1).[20] Next, a one-pot protocol was investigated to prepare the target 1,3-propylene-linked bis(pyrazolo-pyrimidine) **4a** as a typical example. Therefore, a mixture of **2a**, bis(aldehyde) **3a** and malononitrile was subjected to hetero-cyclization under different conditions. The best conditions were found to be heating with piperidine in DMF at 150 °C for 5 h (for the optimization and mechanism of the heterocyclization, see Online Supplementary Materials).[21] Using this protocol, 1*H*-pyrazole-3,5-diamines **2a**–**c** were reacted with different bis(aldehydes) **3a**–**c** and malononitrile to afford the target alkane-linked bis(pyrimidines) **4**–**6** in 85–92% yields (see Scheme 1).



Scheme 1 *Reagents and conditions*: i, $N_2H_4 \cdot H_2O$, EtOH, 80 °C, 3 h; ii, $CH_2(CN)_2$, piperidine, DMF, 150 °C, 5 h, 85–92% yields.



Scheme 2 *Reagents and conditions*: i, $CH_2(CN)_2$, piperidine, DMF, 150 °C, 5 h, 80–86% yields.

Table 1 MIC and MBC values of new bis(pyrazolo[1,5-*a*]-pyrimidines).

Compound	MIC (MBC) / μM			
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
4a	20.7 (41.4)	82.8 (165.7)	20.7 (41.4)	82.8 (165.7)
4b	76.0 (152.0)	152.0 (> 250)	76.0 (152.0)	152.0 (> 250)
4c	9.6 (19.2)	38.4 (76.8)	9.6 (19.2)	38.4 (76.8)
5a	20.3 (40.7)	81.4 (162.9)	20.3 (40.7)	81.4 (162.9)
5b	74.7 (149.5)	149.5 (> 250)	74.7 (149.5)	149.5 (> 250)
5c	9.4 (18.8)	37.7 (75.5)	9.4 (18.8)	37.7 (75.5)
6a	20.0 (40.0)	80.0 (160.0)	20.0 (40.0)	80.0 (160.0)
6b	73.5 (147.0)	147.0 (> 250)	73.5 (147.0)	147.0 (> 250)
6c	9.2 (18.5)	37.1 (74.3)	9.2 (18.5)	37.1 (74.3)
8a	4.7 (9.5)	19.0 (38.0)	4.7 (9.5)	19.0 (38.0)
8b	8.7 (17.5)	35.1 (70.2)	8.7 (17.5)	35.1 (70.2)
8c	2.2 (4.4)	8.8 (17.7)	2.2 (4.4)	8.8 (17.7)
10a	15.1 (30.3)	60.7 (121.5)	15.1 (30.3)	60.7 (121.5)
10b	28.4 (56.9)	113.9 (227.9)	28.4 (56.9)	113.9 (227.9)
10c	7.1 (14.2)	28.7 (57.4)	7.1 (14.2)	28.7 (57.4)
Ciprofloxacin	2.9 (5.9)	2.9 (5.9)	2.9 (5.9)	2.9 (5.9)

Using a similar protocol, we synthesized two new series of *p*-xylene- and thieno[2,3-*b*]thiophene-linked bis(pyrimidines) **8a**–**c** and **10a**–**c** in 80–86% yields utilizing bis(aldehydes) **7** and **9** (Scheme 2).

The new bis(pyrazolo[1,5-*a*]pyrimidines) were screened *in vitro* against strains of *Staphylococcus aureus* (ATCC:6538), *Enterococcus faecalis* (ATCC:29212), *Escherichia coli* (ATCC:9637), and *Pseudomonas aeruginosa* (ATCC:27953). Ciprofloxacin was used as a reference to determine the MIC and MBC values against the selected strains (MIC / MBC values of 2.9 / 5.9 μM) (Table 1).[22–24] Generally, hybrid **8c** showed the best antibacterial activity against all strains tested. It demonstrated stronger activity than ciprofloxacin against *S. aureus* and *E. coli* strains with MIC / MBC values of 2.2 / 4.4 μM. In terms of *E. faecalis*, and *P. aeruginosa* strains, hybrid **8c** had the best efficacy though it showed lower activity than ciprofloxacin with MIC / MBC values of 8.8 / 17.7 μM. Hybrid **8a** was the second in antibacterial activity against all strains tested. It had MIC / MBC values of 4.7 / 9.5 μM against *S. aureus* and *E. coli* strains, whereas it had MIC / MBC values of 19.0 / 38.0 μM against *E. faecalis* and *P. aeruginosa* strains. Other tested hybrids demonstrated decreased efficacy with MIC / MBC values ranging from 7.1 / 152.0 μM against *S. aureus* and *E. coli* strains, whereas they had MIC / MBC values ranging from 28.7 to more than 250 μM against *E. faecalis* and *P. aeruginosa* strains.

In 2022, Hassan *et al.*[25] reported the promising antimicrobial activity of pyrazolo[1,5-*a*]pyrimidines, particularly against *E. coli* and *B. subtilis* strains with inhibition zones of 16 and 21 mm, respectively. The recent focus on the synthesis of bis-heterocyclic hybrids is due to their vital applications in both biology and industry.[26,27] Bis-heterocyclic compounds have also been found to potentially show stronger bioactivity than their mono-analogues.[16,28] The current study was successful in producing a new three-series of bis(pyrazolo[1,5-*a*]pyrimidines) that were tested as potential antibacterial agents. In each series, the two pyrazolopyrimidine units are linked at C5 *via* an appropriate spacer attached to two *p*-phenoxy groups. Additionally, each series is attached to a substituted benzyl unit at C3. The previous benzyl group is attached to one of three *para*-substituents (X) with distinct electronic characteristics.

Taking the antibacterial activity against the *S. aureus* strain as a typical example, we note that the antibacterial activity of propane-linked **4a**–**c** hybrids is related to the electronic properties of the substituent in phenyl group. Table 1 demonstrates that hybrid **4a** with Ar = Ph had moderate activity with MIC / MBC values of 20.7 / 41.4 μM. Incorporating an electron withdrawing substituent (Ar = 4-ClC_6H_4) into hybrid **4b** resulted in reducing the obtained activity with MIC / MBC values of 76.0 / 152.0 μM.

Adding an electron donating substituent (Ar = 4-$MeOC_6H_4$) to the target **4c**, on the other hand, resulted in nearly two-fold more potent activity (MIC/MBC values of 9.6/19.2 μM) than that of **4a**.

The incorporation of an elongated alkane spacer to the target products in butane-linked series **5** and pentane-linked series **6** was also investigated. As shown in Table 1, we observed that varying the alkane linker length had a small influence on the obtained potency. Thus, each hybrid in series **5** or **6** exhibited comparable activity to its analogue in series. Moreover, the same dependence of the activity on the electronic properties of substituent in phenyl group was observed in each series. Hybrids **5c** and **6c** with 4-$MeOC_6H_4$ substituent had the highest activity with MIC and MBC values in the ranges 9.2–9.4 and 18.5–18.8 μM, whereas hybrids **5b** and **6b** with 4-ClC_6H_4 had the least activity with MIC and MBC values in the ranges 73.5–74.7 and 147.0–149.5 μM, respectively.

As for series of *p*-xylene and thieno[2,3-*b*]thiophene-linked bis(pyrimidines) **8** and **10**, they demonstrated superior antibacterial activity over alkane-linked series **4**–**6**. Regarding series **10**, hybrid **10a** with Ph group showed more potent activity than its analogues in series **4**–**6** with MIC/MBC values of 15.1/30.3 μM. Moreover, hybrid **10c** with 4-$MeOC_6H_4$ substituent had improved activity compared to **10a** with MIC/MBC values of 7.1/14.3 μM, whereas hybrid **10b** with 4-ClC_6H_4 had lower activity with MIC/MBC values of 28.4/56.9 μM. Finally, *p*-xylene-linked bis(pyrimidines) **8** demonstrated the best activity among all new products. As shown in Table 1, hybrid **8a** (Ph) had stronger activity than its analogues in series **4**–**6** or **10** with MIC/MBC values of 4.7/9.5 μM. Moreover, hybrid **8c** (4-$MeOC_6H_4$) exhibited the highest activity with MIC/MBC values of 2.2/4.4 μM, whereas hybrid **8b** (4-ClC_6H_4) had lower activity than **8a** with MIC and MBC values of 8.7/17.5 μM.

To sum up, new bis(pyrazolo[1,5-*a*]pyrimidines) linked to different spacers by *p*-phenoxy units were efficiently prepared by reacting a ternary mixture of the appropriate 1*H*-pyrazole-3,5-diamines, malononitrile and bis(aldehydes). The obtained antibacterial activity of new hybrids is influenced by the respective spacer at C5 as well as the *para*-substituted benzyl unit at C3. The *p*-xylene-linked hybrids bearing 3-positioned 4-methoxybenzyl substituents showed the best activity.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.04.011.

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