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Synthesis and In Silico Screening of Biological Activity of Novel Bisazomethines Containing 1,3-Diazine Fragment: Perspectives in Organic Synthesis and Pharmaceuticals †

Aleksandr V. Dambaev *, Denis A. Kolesnik, Igor P. Yakovlev, Tamara L. Semakova and Polina O. Levshukova

State Federal-Funded Educational Institution of Higher Education "Saint Petersburg State Chemical and Pharmaceutical University" of the Ministry of Healthcare of the Russian Federation, Professor Popov Str., 14, Lit. A, St. Petersburg 197022, Russia; denis.kolesnik@spcpu.ru (D.A.K.); igor.yakovlev@pharminnotech.com (I.P.Y.); tamara.semakova@pharminnotech.com (T.L.S.); levshukova.polina@pharminnotech.com (P.O.L.)

* Correspondence: aleksandr.dambaev@spcpu.ru

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Abstract

Bridged 1,3-diazine bisazomethines were synthesized via nucleophilic addition of phenylenediamines to 4,6-dihydroxypyrimidine-5-carbaldehydes in methanol/water. Structures were confirmed by NMR spectroscopy. In silico screening (PASS-online, CLC-pred, Antivir-pred, GUSAR-online) revealed broad bioactivity (antibacterial, antifungal, antiviral) and OECD Class 4 toxicity, indicating high safety and therapeutic potential.

Keywords: bisazomethines; nucleophilic addition; schiff bases; pyrimidine-5-carbaldehyde

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1. Introduction

The pyrimidine heterocycle, being a key pharmacophore, continues to be a focus of intensive research in medicinal chemistry. In particular, hybrid structures that combine a pyrimidine core with an azomethine fragment have attracted significant interest due to their broad spectrum of biological activities [1–4]. However, bisderivatives based on functionalized pyrimidine aldehydes remain poorly studied.

The conjugation of two or more pharmacophoric fragments into a single molecular architecture is a powerful strategy for creating new chemical entities with promising biological properties. We assumed that 4,6-dihydroxy-2-methylpyrimidine-5-carbaldehydes could serve as an ideal platform for constructing such systems. The one-step approach we propose enables the efficient synthesis of complex, polyfunctional compounds from readily available starting materials.

The aim of the present work was to develop a synthetic method for novel, previously unreported bisazomethines based on 4,6-dihydroxypyrimidine-5-carbaldehydes and to confirm their structures. The potential of the synthesized compounds as candidates for pharmacological studies was indirectly supported by preliminary in silico screening.

2. Materials and Methods

The potential biological profiles of the target compounds were evaluated through in silico screening. A broad spectrum of activities was predicted using the PASS-online [5], while more focused assessments of antitumor and antiviral potential were conducted with the CLC-pred and Antivir-pred tools, respectively. Prospective acute toxicity was estimated using the GUSAR-online service.

The target bisazomethines—5,5′-{1,3-phenylenebis[azanylidenemethylylidene]}bis(2-methylpyrimidine-4,6-diol) (**3**), 5,5′-{1,4-phenylenebis[azanylidenemethylylidene]}bis(2-methylpyrimidine-4,6-diol) (**4**), 5,5′-{1,3-phenylenebis[azanylidenemethylylidene]}bis(pyrimidine-4,6-diol) (**5**), and 5,5′-{1,4-phenylenebis[azanylidenemethylylidene]}bis(pyrimidine-4,6-diol) (**6**)—were synthesized by condensing 4,6-dihydroxypyrimidine-5-carbaldehyde (**1**) or 4,6-dihydroxy-2-methylpyrimidine-5-carbaldehyde (**2**), prepared according to a known procedure [6], with the corresponding bisnucleophilic component (benzene-1,3-diamine or benzene-1,4-diamine) in a 2:1 molar ratio. The reactions were performed in either aqueous or absolute methanolic medium with the addition of glacial acetic acid (Figure 1).

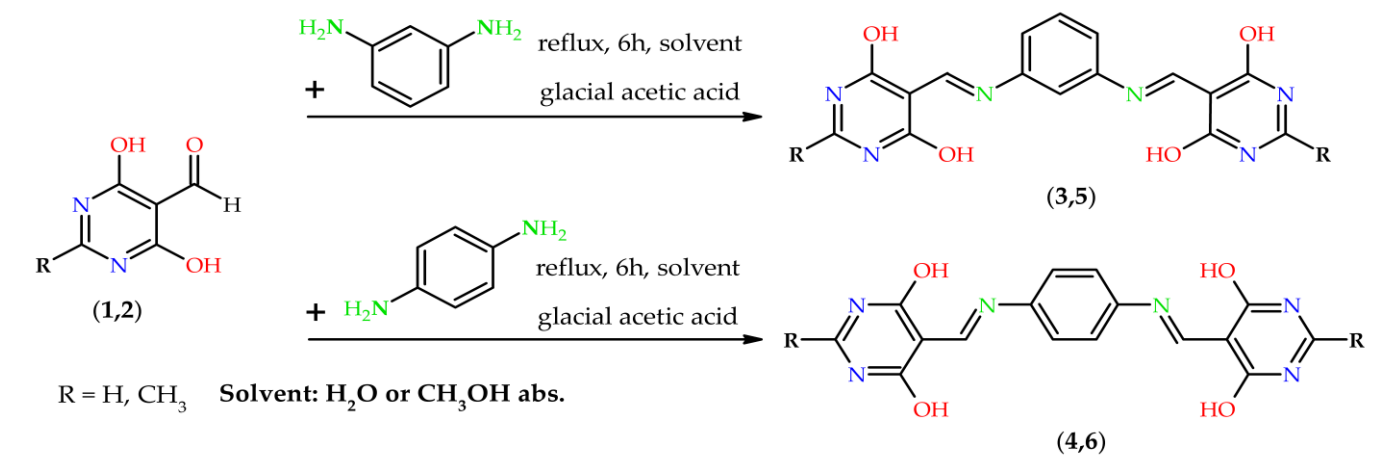

Figure 1. Synthesis scheme of bisazomethine derivatives **3**–**6**.

The reaction progress was monitored by thin-layer chromatography (TLC) by tracking the disappearance of the corresponding phenylenediamine. A mixture of methanol, dichloromethane, and hexane (1:9:1) was used as the mobile phase. Detection was performed under UV-light at a wavelength of 254 nm. The structures of the synthesized compounds were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

*Synthesis of 5,5′-{Phenylenebis[azanylylenemethylidenyl]}bis(pyrimidine-4,6-diols) (**3**–**6**)*

Preparation of bisazomethine derivatives **3**–**6**: In a typical experiment, the corresponding carbaldehyde (**1** or **2**, 3.14 mmol) was suspended in 25 mL of either absolute methanol or water. Benzene-1,4-diamine or its 1,3-isomer (1.57 mmol) and glacial acetic acid (0.8 mmol) were introduced to the suspension. The system was equipped with a reflux condenser and heated for 6 h with constant stirring. The consumption of the aromatic diamine was tracked by TLC. Upon completion, the precipitated product was filtered off, providing compounds **3**–**6**. Isolated yields are compiled in Table 1.

Table 1. Yields of products **3**–**6**.

Compound Number	3	4	5	6
Yield in water (%)	83	85	87	84
Yield in methanol (%)	88	90	93	91

The structure of the compounds was confirmed by NMR spectroscopy using a Bruker AM-600 (PubCompare, Switzerland). Compounds **3**, **4**, **5**, **6** were analyzed solely in deuterated trifluoroacetic acid.

Nuclear magnetic resonance spectrum data ^{1}H (400 MHz, TFA-d), δ, ppm, of product **3** (Figure 2): 3.21 s (5.98H, -CH_3), 8.03 d (2.09 H, H: C4″, C6″), 8.11 t (1.16 H, H: C5″), 8.37 s (1.02 H, H: C2″), 9.62 s (2.08 H, -N=CH-).

Nuclear magnetic resonance spectrum data ^{13}C (100 MHz, TFA-d), δ, ppm, of product **3**: 12.84 (-CH3), 90,98 (C–5, 5′), 104.11 (C–2″), 116.21 (C–4″, 6″),127.11 (C–5″), 135,63 (C–1″, 3″), 149.65 (C–2, 2′), 154.23 (C–4, 4′, 6, 6′), 161.91 (-N=CH-).

Nuclear magnetic resonance spectrum data ^{1}H (400 MHz, TFA-d), δ, ppm, of product **5**: 8.06 d (2.09 H, H: C4″, C6″), 8.15 t (1.16 H, H: C5″), 8.40 s (1.02 H, H: C2″), 8.93 s (1.97 H, H: C2, C2′), 9.69 s (2.08 H, -N=CH-).

Nuclear magnetic resonance spectrum data ^{13}C (100 MHz, TFA-d), δ, ppm, of product **5**: 90,08 (C–5, 5′), 104.11 (C–2″), 116.41 (C–4″, 6″),127.71 (C–5″), 135,83 (C–1″, 3″), 149.78 (C–2, 2′), 153.34 (C–4, 4′, 6, 6′), 161.48 (-N=CH-).

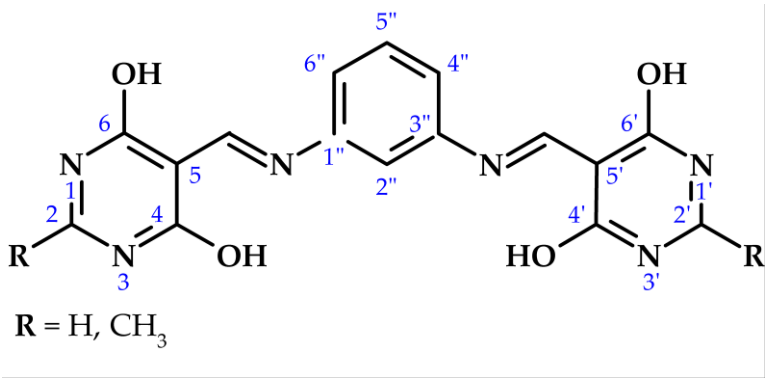

Figure 2. Structural formula of 5,5′-{1,3-phenylenebis[azanylylenemethylidenyl]} bis(pyrimidine-4,6-diols) (**3**,**5**).

Nuclear magnetic resonance spectrum data ^{1}H (400 MHz, TFA-d), δ, ppm, of product **4** (Figure 2): 3.18 s (6.00 H, -CH3), 8.11 s (4.21 H, H-Ar), 9,50 s (1.85 H, -N=CH-).

Nuclear magnetic resonance spectrum data ^{13}C (100 MHz, TFA-d), δ, ppm, of product **4**: 11.46 (-CH3), 88.75 (C–5, 5′), 115.21 (C–2″, 3″, 5″, 6″), 130.56 (C–1″, 4″), 149.95 (C–2, 2′), 155.99 (C–4, 4′, 6, 6′), 160.87 (-N=CH-).

Nuclear magnetic resonance spectrum data ^{1}H (400 MHz, TFA-d), δ, ppm, of product **6**: 8.14 s (4.00 H, H-Ar), 8.65 s (2.01 H: C 2, 2′), 9.62 s (1.89 H, -N=CH-).

Nuclear magnetic resonance spectrum data ^{13}C (100 MHz, TFA-d), δ, ppm, of product **6**: 89.01 (C–5, 5′), 116.11 (C–2″, 3″, 5″, 6″), 131.96 (C–1″, 4″), 148.15 (C–2, 2′), 155.91 (C–4, 4′, 6, 6′), 161.03 (-N=CH-).

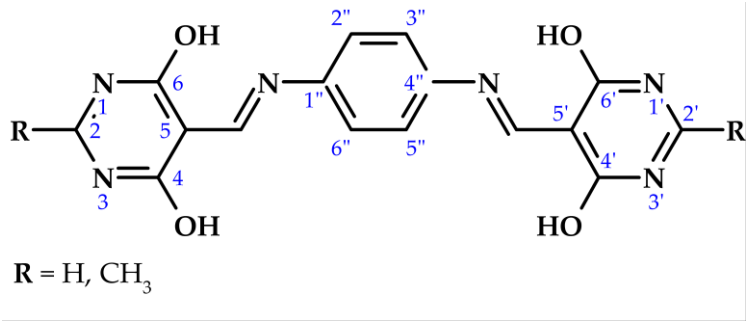

Figure 3. Structural formula of 5,5′-{1,3-phenylenebis[azanylylenemethylidenyl]} bis(pyrimidine-4,6-diols) (**4**,**6**).

3. Results

According to screening results obtained via the web resources PASS Online, CLC-pred, and Antivir-pred, the synthesized compounds demonstrate a high likelihood of exhibiting antihypertensive effects, activity targeting cisplatin-resistsant ovarian carcinoma, and antiviral efficacy activity against influenza virus (Table 2).

Table 2. Results of in silico biological activity screening.

Web Resource	Activity	Predicted Probability (Pa)
PASS online	Antihypertensive	0.7
CLC-pred	Activity against cisplatin-resistsant ovarian carcinoma	0.7
Antivir-pred	anti-influenza activity	0.2

The in silico assessment of acute toxicity, performed using the GUSAR online platform, categorized the synthesized compounds under toxicity class 4 for intravenous, oral, and subcutaneous routes of administration. In contrast, a classification of toxicity class 5 was predicted for intraperitoneal injection (Table 3).

Table 3. Predicted LD50 values depending on route of administration in mg/kg with preliminary assignment to OECD * toxicity classes.

Intraperitoneal	Intravenous	Oral	Subcutaneous
530	178	1801	856
Class 5	Class 4	Class 4	Class 4

* The organisation for economic co-operation and development.

From the data presented in the table above, it can be concluded that these compounds potentially exhibit a high safety profile.

The target compounds **3**, **4**, **5** and **6** were obtained by condensation reaction between pyrimidine-5-carbaldehydes (**1**, **2**) and *o*- or *p*-phenylenediamine. The use of absolute methanol as the solvent increases the yield of the target product compared to water. This effect is likely due to the higher solubility of the starting materials in absolute methanol. Their structure was reliably proved by ^{1}H and ^{13}C NMR spectroscopy.

The structural assignment of product **4** was supported by its ^{1}H NMR spectrum (TFA-d, Figure 4). Diagnostic resonances comprise a singlet at 9.50 ppm (integrated intensity 1.85) for the azomethine (CH=N) protons and a singlet at 3.18 ppm (integrated intensity 6.00) for the methyl groups attached to the pyrimidine cores. Additionally, the aromatic protons of the p-phenylene bridge give rise to a set of signals at 8.11 ppm (integrated intensity 3.81).

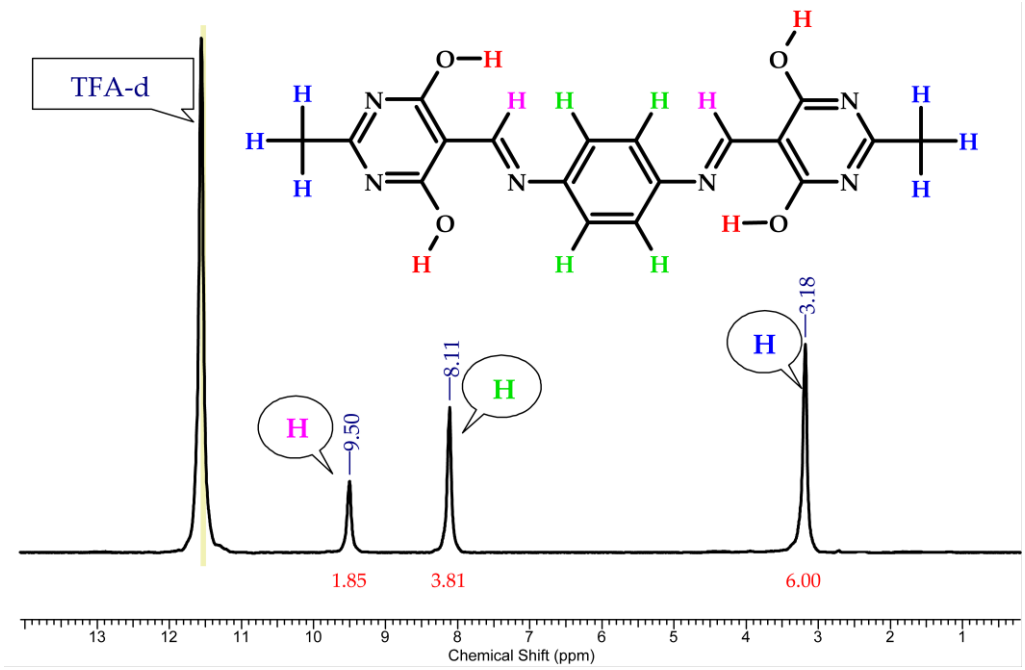

Figure 4. [1]H NMR spectrum of 5,5′-{1,4-phenylenebis[azanylylenemethylidenyl]}bis (2-methylpyrimidine-4,6-diol).

As illustrated in Figure 5, the [13]C NMR spectrum (TFA-d) of product **4** provided full assignment of the carbon skeleton. Key resonances were assigned to the following structural motifs: the azomethine carbons at 160.87 ppm; the hydroxyl-substituted pyrimidine carbons (C–4, C–4′, C–6, C–6′) at 155.99 ppm; the *p*-phenylene bridge carbons (C–1″, C–4″: 130.56 ppm; C–2″, C–3″, C–5″, C–6″: 115.21 ppm); the C–2, C–2′ and C–5, C–5′ pyrimidine carbons at 88.75 ppm; and the methyl carbons at 11.46 ppm.

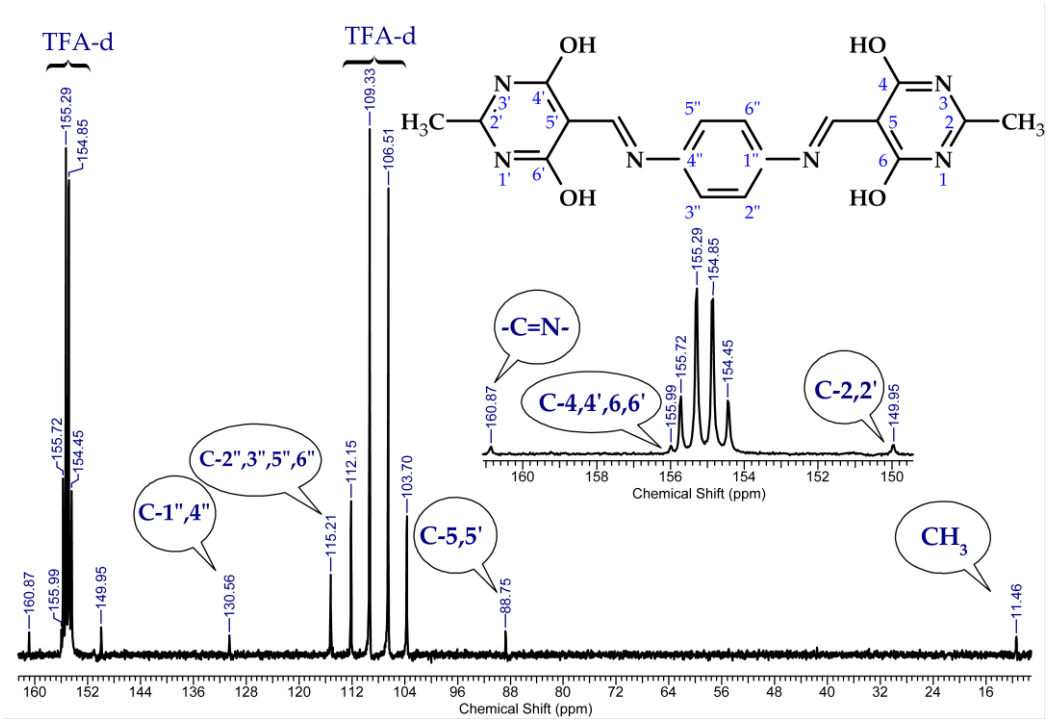

Figure 5. [13]C NMR spectrum of 5,5′-{1,4-phenylenebis[azanylylenemethylidenyl]}bis(2-methylpyrimidine-4,6-diol).

4. Conclusions

The studied bisazomethine derivatives exhibit a high probability of possessing a broad spectrum of biological activity, particularly antihypertensive, antitumor, and antiviral, as predicted by the PASS online, CLC-pred, and Antivir-pred web resources.

The condensation of 4,6-dihydroxypyrimidine-5-carbaldehyde precursors with *o*- and *p*-phenylenediamines (2:1 molar ratio) provided direct access to previously unreported bisazomethine systems. Reaction optimization studies identified absolute methanol as the optimal medium, consistently furnishing the target molecules in high yields of 80-90%, markedly outperforming aqueous conditions. The significance of these bisazomethines is twofold: their inherent molecular architecture suggests potential for diverse biological activities, while their functional group richness positions them as privileged scaffolds for subsequent medicinal chemistry exploration.

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