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Pseudo-four-component synthesis of 5-(4-hydroxy-2-oxo-1,2-dihydropyridin-3-yl)-substituted 5*H*-chromeno[2,3-*b*]pyridines and estimation of its affinity to sirtuin 2

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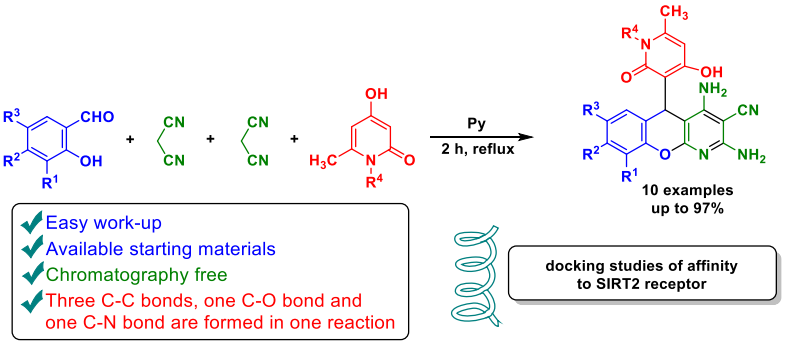
Abstract

Multicomponent reactions (MCRs) are masterpieces of synthetic efficiency and reaction design which allow the rapid generation of complex molecules with biologically relevant scaffold fragments. A new pseudo-four-component, one-pot synthetic scheme yielding 5-(4-hydroxy-2-oxo-1,2-dihydropyridin-3-yl)-substituted 5*H*-chromeno[2,3-*b*]-pyridines in 40-97% yields is reported. This 'one-pot' process opens an efficient and convenient way to substituted 5H-chromeno[2,3-b]pyridines, which are promising compounds for biomedical applications. Molecular docking studies of the synthesized 5H-chromeno[2,3-*b*]pyridines were carried out to identify their relationship with the binding pockets of sirtuin 2 (SIRT2).



Keywords: Multicomponent reactions, salicylaldehydes, 6-hydroxy-4-methylpyridin-2(1*H*)-ones, malononitrile, 5*H*-chromeno[2,3-*b*]pyridines, docking studies

Introduction

Multicomponent reactions (MCRs) are masterpieces of synthetic efficiency and reaction design.[1] MCRs are flexible reactions for the rapid generation of complex molecules with biologically relevant scaffold fragments.[2] This strategy provides high efficiency, operational simplicity and low waste formation.[3] The multicomponent methodology is based on the pot-economy principle, and unites it with the atom- and step-economy concepts.[4]

Neurodegeneration is a process which leads to irreversible neuronal damage and death, and is a common final pathway present in aging and neurodegenerative diseases.[5] Neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, are among the greatest challenges the world faces this century.[6] One new case of neurodegenerative disease, most commonly dementia, appears every three seconds in the world. There are studies in cell and invertebrate models of Parkinson's disease and Huntington's disease which have proposed potential neuroprotective effects of SIRT2 (sirtuin 2) inhibition.[7] SIRT2 is a NAD-dependent deacetylase, involved in the regulation of the cell cycle, tumorigenesis and other important processes related to metabolic homeostasis.[8]

In recent years, the notion of 'privileged scaffolds' has become one of the main ideas in the search for new drugs.[9] These privileged scaffolds have semi-rigid heterocyclic systems which determine the orientation type of different functional substituents in order to be recognized by the target molecule.

The chromeno[2,3-*b*]pyridine moiety is a well-known scaffold in medicinal chemistry. Most of its derivatives possess a wide spectrum of pharmacological properties, such as glucocorticoid-receptor (GR) agonists,[10] antiproliferative,[11] anti-tumor,[12] and anti-asthmatic.[13] Amlexanox (Fig. 1) is an anti-allergic drug which is most often used in the treatment of rhinitis and asthma.[13] It is also an effective remedy for recurrent canker sores.[14] Pranoprofen (Fig. 1) is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties.[15] Chromenotacrine CT6 (Fig. 1) is a non-toxic antioxidant and neuroprotective agent.[16] Some substituted *5H*-chromeno[2,3-*b*]pyridines (Fig. 1) inhibit mitogen-activated protein kinase 2 (MK-2) and are able to suppress expression of TNFα in U937 cells as anti-rheumatoid and anti-psoriatic agents.[17]

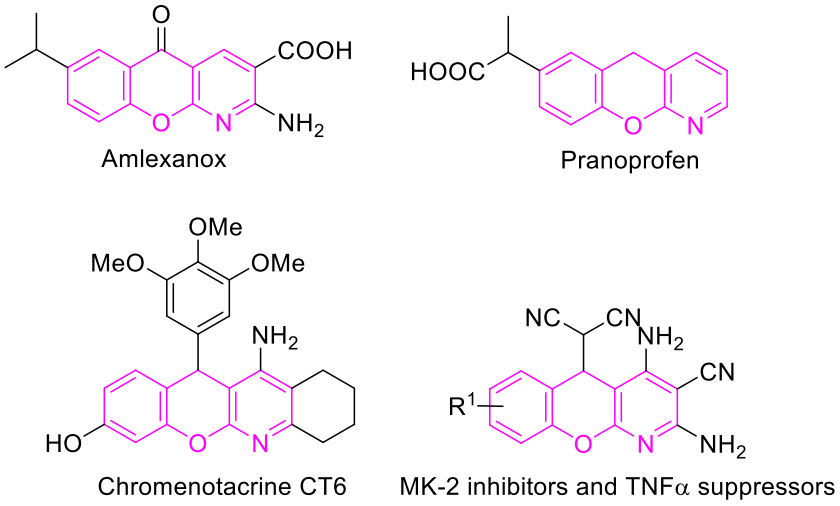
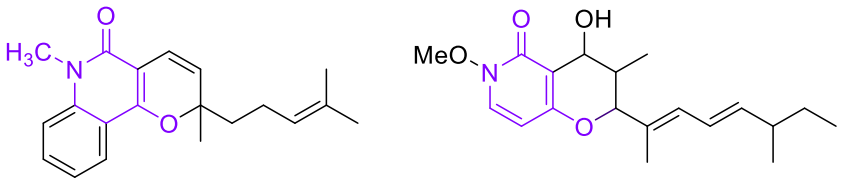


Figure 1. Medicinal interest in molecules with the *5H*-chromeno[2,3-*b*]pyridine scaffold

The pyridin-2(1*H*)-one fragment is also a widely known medicinal scaffold. It has been found in different alkaloids and other natural compounds. Zanthosimuline (Fig.2) was isolated from fruits or root bark and used in the treatment of different drug-resistant cancer types.[18] YCM1008A (Fig.2) is known as a Ca^{2+}-signaling

inhibitor and is able to suppress Ca^{2+}-induced growth inhibition of the *Saccharomyces cerevisiae* mutant.[19] The pyridin-2(1*H*)-one moiety is part of a natural nucleoside that is involved in bio-membrane synthesis[20] and galactose metabolism,[21] and plays an important role in the regulation of body temperature,[22] and in peripheral and central nervous system activity.[23]



Zanthosimuline YCM1008A

Figure 2. Bioactive molecules with pyridin-2(1*H*)-one moiety

These diverse therapeutic activities of chromeno[2,3-*b*]pyridines and pyridin-2(1*H*)-one derivatives explain the interest in the combination of both of these fragments.

In recent years, we have accomplished multicomponent syntheses of new heterocyclic systems using different types of 'one-pot' transformations of carbonyl compounds and C-H acids.[24-27] Recently, we carried out multicomponent transformations of salicylaldehydes, 2-aminoprop-1-ene-1,1,3-tricarbonitrile and 3-phenylisoxazol-5(4*H*)-one,[28] 3-methyl-2-pyrazolin-5-one[29] and 6-hydroxy-4-methylpyridin-2(1*H*)-ones.[30] Furthermore, we have implemented multicomponent reactions of salicylaldehydes, two equivalents of malononitrile and 1,3-cyclohexanediones.[31]

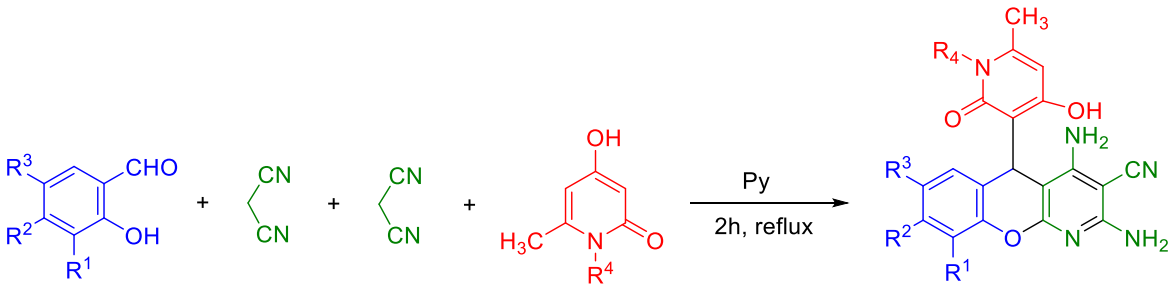
Taking into consideration our previous results, and the scientific relevance of chromeno[2,3-*b*]pyridines and pyridin-2(1*H*)-ones, we wished to develop a methodology for the efficient pseudo-four-component transformation of salicylaldehydes (**1a-g**), malononitrile and 6-hydroxy-4-methylpyridin-2(1*H*)-ones (**2a,b**) into 5-(4-hydroxy-2-oxo-1,2-dihydropyridin-3-yl)-substituted *5H*-chromeno[2,3-*b*]pyridines (**3a-j**) (Scheme 1).

Results and Discussion

Multicomponent syntheses without organic solvents are among the important challenges of modern green chemistry.[32] Thus, solvent-free[33,34] and in-water reactions have been investigated intensively in recent years.[35,36] Here we report our results on the novel pseudo-four-component transformation of salicylaldehydes (**1a-g**), malononitrile and 6-hydroxy-4-methylpyridin-2(1*H*)-ones (**2a,b**) into 5-(4-hydroxy-2-oxo-1,2-dihydropyridin-3-yl)-substituted *5H*-chromeno[2,3-*b*]pyridines (**3a-j**). (Scheme 1, Tables 1 and 2).

We started with the synthesis of earlier unknown 5*H*-chromeno[2,3-*b*]pyridine (**3a**) from salicylaldehyde (**1a**), malononitrile (two equivalents) and 6-hydroxy-4-methyl-pyridin-2(1*H*)-one (**2a**) under solvent-free and aqueous conditions (entries 1-5, Table 1). Under these conditions, the yields of 5*H*-chromeno[2,3-*b*]pyridine (**3a**) were only within the range of 8-23%.

In ethanol, without a catalyst and with different catalysts, the yields of 5*H*-chromeno[2,3-*b*]pyridine (**3a**) were increased to 32-64% (entries 6-10, Table 1). A significant increase in the yield (up to 93%) was achieved when the reaction was conducted in pyridine as a solvent and catalyst (entries 11-13, Table 1). When we carried out the transformation in *n*-propanol or bromobenzene, the reaction yield did not increase (entries 14-17, Table 1). Following refluxing of the salicylaldehydes, malononitrile and 6-hydroxy-4-methylpyridin-2(1*H*)-ones in pyridine for 2 h, the optimum conditions, 5*H*-chromeno[2,3-*b*]pyridine (**3a**) was isolated in 92% yield (entry 12, Table 1).



1a-f

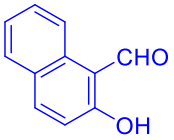
a $R^1 = R^2 = R^3 = H$
b $R^1 =$ OEt, $R^2 = R^3 = H$
c $R^1 = R^3 = H$, $R^2 =$ OMe
d $R^1 = R^2 = H$, $R^3 = Cl$
e $R^1 = R^3 = I$, $R^2 = H$
f $R^1 = R^2 = H$, $R^3 = NO_2$

2a,b

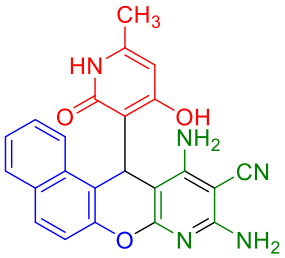
a $R^4 = H$
b $R^4 = CH_2Ph$

3a-f, h-j

a $R^1 = R^2 = R^3 = R^4 = H$
b $R^1 =$ OEt, $R^2 = R^3 = R^4 = H$
c $R^1 = R^3 = R^4 = H$, $R^2 =$ OMe
d $R^1 = R^2 = R^4 = H$, $R^3 = Cl$
e $R^1 = R^3 = I$, $R^2 = R^4 = H$
f $R^1 = R^2 = R^4 = H$, $R^3 = NO_2$
h $R^1 = R^2 = R^3 = H$, $R^4 = CH_2Ph$
i $R^1 = R^3 = H$, $R^2 =$ OMe, $R^4 = CH_2Ph$
j $R^1 = R^2 = H$, $R^3 = Cl$, $R^4 = CH_2Ph$



1g



3g

Scheme 1. Multicomponent transformation of salicylaldehydes (**1a-g**), malononitrile and 6-hydroxy-4-methyl-pyridin-2(1*H*)-ones (**2a, b**) into 5-(4-hydroxy-2-oxo-1,2-dihydropyridin-3-yl)-substituted 5*H*-chromeno-[2,3-*b*]pyridines (**3a-j**)

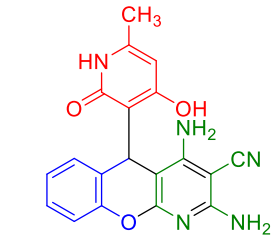
Under the optimal conditions, the 5-(4-hydroxy-2-oxo-1,2-dihydropyridin-3-yl)-substituted 5*H*-chromeno[2,3-*b*]pyridines (**3a-j**) were obtained in 40-97% yields (Table 2). Isolation of the final compounds is an easy work-up procedure and does not require any further purification steps. After the reactions were finished, the solids were filtered, washed with chilled ethanol, and dried to isolate the pure 5*H*-chromeno[2,3-*b*]pyridines (**3a-j**).

Table 1. Multicomponent transformation of salicylaldehyde (**1a**), two equivalents of malononitrile and 6-hydroxy-4-methylpyridin-2(1*H*)-one (**2a**) into *5H*-chromeno[2,3-*b*]pyridine (**3a**)

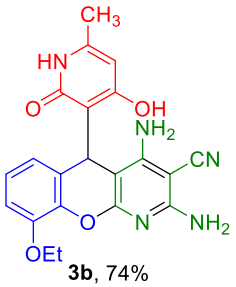
Entry	Solvent	Catalyst	Temperature, °C	Time, h	Yield of **3a** (%)
1	Solvent-free	-	60	1	8[a]
2	Solvent-free	NaOAc	60	1	12[a]
3	Solvent-free	KF	60	1	10 [a]
4	H_2O	-	80	1	12[a]
5	H_2O	NaOAc	80	1	23[a]
6	EtOH	-	78	1	32[a]
7	EtOH	NaOAc	78	1	52[a]
8	EtOH	NaOH	78	1	59[a]
9	EtOH	Et_3N	78	1	61
10	EtOH	Py	78	1	64
11	Py	-	115	1	77
12	Py	-	115	2	92
13	Py	-	115	4	93
14	PhBr	-	156	2	32[a]
15	PhBr	NaOAc	156	2	50[a]
16	*n*-PrOH	-	97	2	55[a]
17	*n*-PrOH	NaOAc	97	2	75

Reaction conditions: salicyaldehyde (**1a**) (3 mmol), malononitrile (6 mmol) and 6-hydroxy-4-methyl-pyridin-2(1*H*)-one (**2a**) (3 mmol) were heated in 5 mL of solvent or without solvent; with 10 mol% of catalyst or without catalyst. [a]Yield by NMR data.

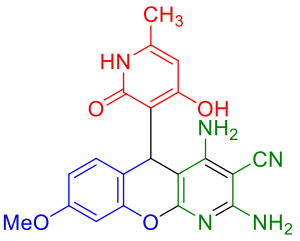
Table 2. Substituted 5*H*-chromeno[2,3-*b*]pyridines (**3a-j**) formed by pseudo-four-component assembly[a]



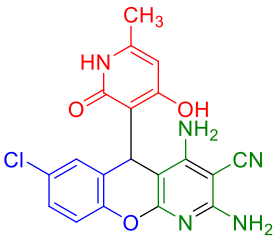
3a, 92%



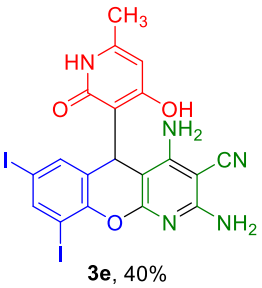
3b, 74%



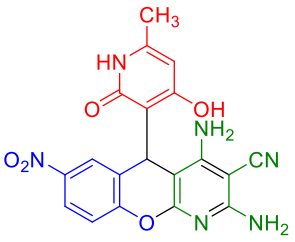
3c, 87%



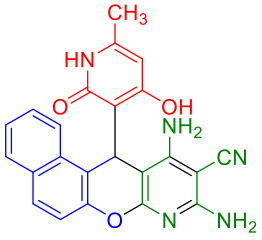
3d, 70%



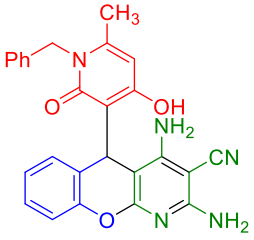
3e, 40%



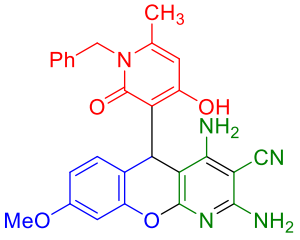
3f, 60%



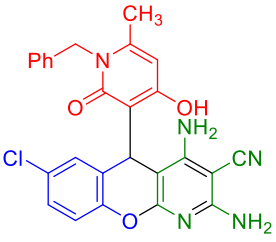
3g, 97%



3h, 80%



3i, 88%



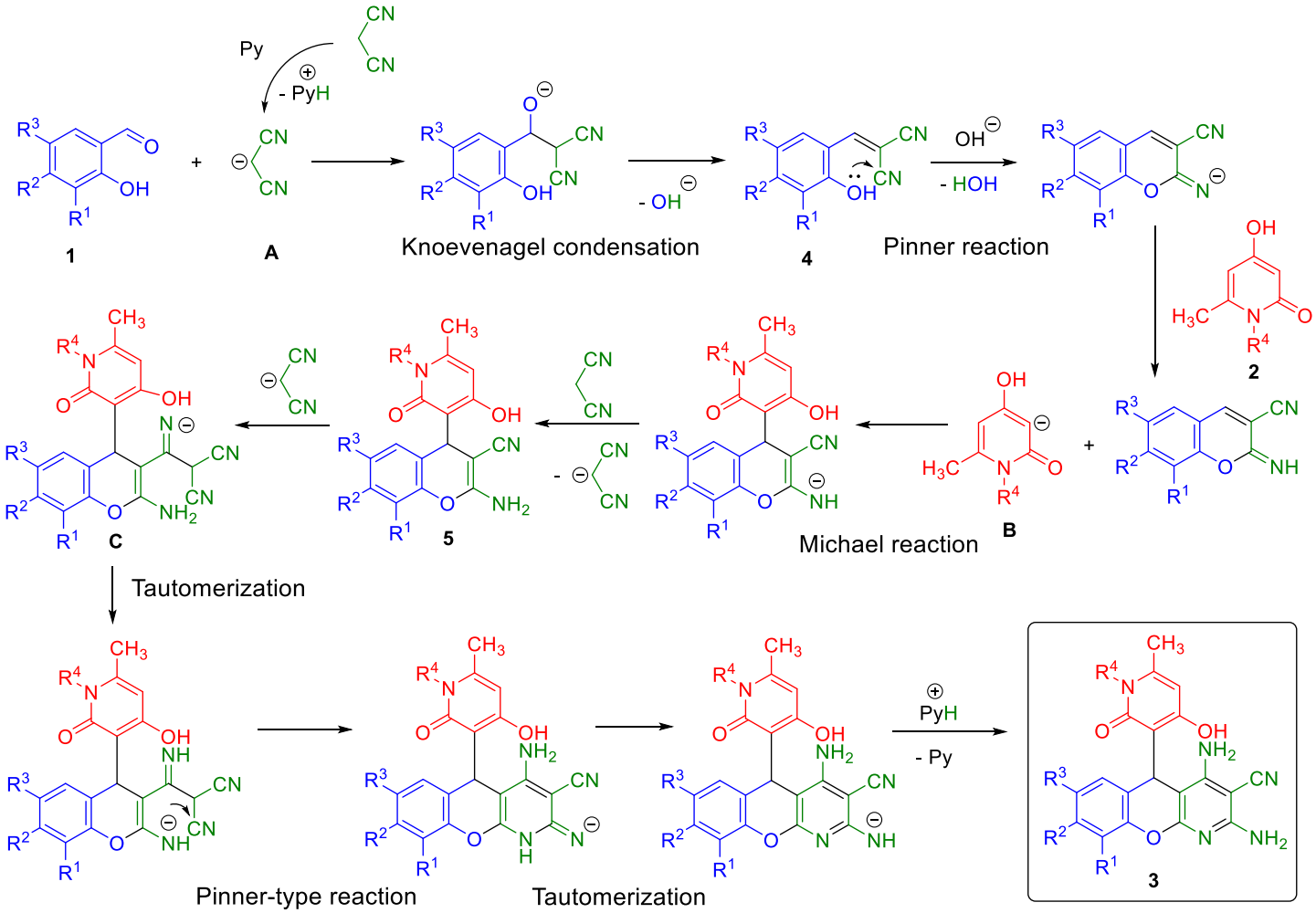
3j, 51%

Reaction conditions: salicyaldehydes (**1a-g**) (3 mmol), malononitrile (6 mmol), 6-hydroxy-4-methylpyridin-2(1*H*)-one (**2a,b**) (3 mmol) were refluxed in 5 mL of pyridine for 2 h. [a] Isolated yields.

The structures of 5*H*-chromeno[2,3-*b*]pyridines (**3a-j**) were confirmed by NMR, IR and mass spectrometry data. The structures of new compounds (**3b-e**,**i**,**j**) were additionally confirmed by high resolution mass-spectrometry data.

With all of the above mentioned data, and the results of assembly of the *5H*-chromeno[2,3-*b*]pyridine scaffold from carbonyl compounds and C-H acids,[31,37,38] the following mechanism for the multicomponent

transformation of salicylaldehydes (**1**), two equivalents of malononitrile, and 6-hydroxy-4-methylpyridin-2(1*H*)-ones (**2**) into 5*H*-chromeno[2,3-*b*]pyridines (**3**) is proposed in Scheme 2.



Scheme 2. Mechanism of multicomponent transformation of salicylaldehydes (**1**), two equivalents of malononitrile and 6-hydroxy-4-methylpyridin-2(1*H*)-ones (**2**) into 5*H*-chromeno[2,3-*b*]pyridines (**3**)

In the first step, the formation of malononitrile anion (**A**) takes place by the action of pyridine. The reaction of anion (**A**) with salicylaldehyde (**1**) results in the Knoevenagel adduct formation (**4**) with the elimination of hydroxide anion.[39] The subsequent intramolecular Pinner cyclization of adduct (**4**), followed by the Michael addition of 4-methylpyridin-2(1*H*)-one anion (**B**) leads to 4*H*-chromene-3-carbonitrile (**5**) with regeneration of malononitrile anion, which then attacks the nitrile group in the pyrane ring of the 4*H*-chromene-3-carbonitrile, resulting in the formation of anion (**C**). Further Pinner-type cyclization of anion (**C**), followed by tautomerization and protonation, leads to *5H*-chromeno[2,3-*b*]pyridine (**3**). 4*H*-Chromene-3-carbonitrile (**5**) was isolated in 82% yield following refluxing of the starting reagents in pyridine for 0.5 h.

Docking studies

Molecular docking has become a powerful approach for structure-based drug discovery as the docking programs are able to provide correct predictions.[40] Docking aims to predict a correct pose (binding mode) for a ligand in the binding pocket, and to assess/score affinity (binding energy) for that pose. SIRT2 was predicted as a possible target for compounds **3a-j** by PASS service.[41-43] We were prompted to investigate the compatibility of the synthesized compounds **3a-j** to SIRT2 to estimate the applicability of such a prediction method.

Initially, PDB 5Y0Z[44] was used as the target protein which corresponds to the 3D structure of Human SIRT2 in a complex with a specific inhibitor. The results of docking procedure for **3a-j** are presented in Table 3 in the Supplementary Material (SM) available on the publisher's website. The best binding affinity, -9,283 kcal/mol, was shown by *5H*-chromeno[2,3-*b*]pyridine (**3h**). That seems to be a good result corresponding to the initial PASS prediction. The worst affinity, -3,157 kcal/mol, was calculated for **3b**, Evdw Scoring was within -12,755 to -29,242 kcal/mol, evidencing hydrophobic interaction prevalence. For **3h**, a good -41,234 Emodel Score was assessed (the lower the better). Surprisingly, positive Emodel scores were calculated for compounds **3a, 3b, 3e, 3f, 3g, 3j**. Such a strange distribution of Emodel Score was explained by distortions in a specific crystal structure, which prompted us to investigate another 5YQL crystal structure.[45]

The results for docking procedures of **3a-j** to the 5YQL binding pocket are presented in Table 4 (SM). The binding modes were calculated only for **3f** and **3h-3j**. The binding mode of **3f** was ranked as the most plausible with a -100,296 score, but its affinity was only -2,594 kcal/mol. The best G Score and Emodel Score were assessed for **3h**, again. Despite the fact that several compounds had no binding modes, docking to 5YQL showed the tendency of *5H*-chromeno[2,3-*b*]pyridine derivatives such as **3f** and **3h** to have good binding modes in the binding pocket.

Docking procedure for **3a-j** to 5DY5 crystal structure[46] resulted in good binding modes for each compound with the exception of **3d and g**, the Emodel Score was below -30 for each compound (Table 5 in SM). The best binding affinity, -4,450 kcal/mol, was calculated for **3h**. The others were within the range of -1,364 to -3,771 kcal/mol. Thus, good binding modes into SITR2 were found for other 5*H*-chromeno[2,3-b]pyridines.

The docking studies for crystal structure 5G4C[47] showed good results for each of the compound **3a-j** (Table 6 in SM)**.** The studies showed mostly high binding affinities (-7,871to -11,730 kcal/mol) with exception of **3i** (-6,588 kcal/mol). The Emodel Score and Evdw Score were significantly better than the ones for 5Y0Z, 5YQL, and 5DY5, evidencing increasing binding mode reliability and Van der Waals impact.

Despite the good results, the 5G4C crystal structure contains SIRT2 and the short chain fatty acyl lysine (ligand), which isn't a SIRT2 inhibitor. In addition, we focused on searching for an appropriate crystal structure containing SIRT2 inhibitor into binding pocket. The 4RMG crystal structure was found.[48] Compounds **3a-j** perfectly fitted 4RMG during docking studies (Table 7 in SI). The binding affinity for each compound was above 7 kcal/mol, and the best binding affinity, -11,194 kcal/mol, was assessed for **3h**. In comparison of the results for 5G4C, the Evdw Score slightly decreased, supposedly because of an increase of electrostatic and hydrogen bonding effects. The Emodel Scores for binding modes remained at the high probability level (< +100). As the binding mode visual analysis showed, docking of **3h** to each crystal structure accompanied by anchoring to hydrophobic chains PHE131-LEU138 and ILE232-PHE235, and π-π stacking to PHE96 or PHE119, prevails. Hydrogen bonding increases binding affinity. The most effective hydrogen bond formed to chain GLN167, ASN168, ASP170 (Fig. 3, **3h** interactions). The same pattern fits to the best Emodel Scored into 5YQL (**3f**) and worst scored, **3g** (Fig. 3, **3f, 3g** interactions). The most effective interaction is taking place when *5H*-chromeno[2,3-*b*]pyridine is surrounded by hydrophobic and polar (but not charged) residues. In general, the

anchoring residues of binding site remain the same and without strong dependence on *5H*-chromeno[2,3-*b*]pyridine substituents. Fig. 4 shows the 3D-disposition of compound **3h** into 4RMG binding pockets in helix and surface views.

Thus, PASS service provides an interesting hint at the biological activity of the synthesized compounds. Distinct protein crystal structures could depend on their preparation, hence, several docking studies for distinct crystal structures of the same protein have been carried out to shed some light on what prevents docking of potential ligands. *5H*-chromeno[2,3-*b*]pyridine (**3h**) showed good affinity to SIRT2 for several crystal structures.

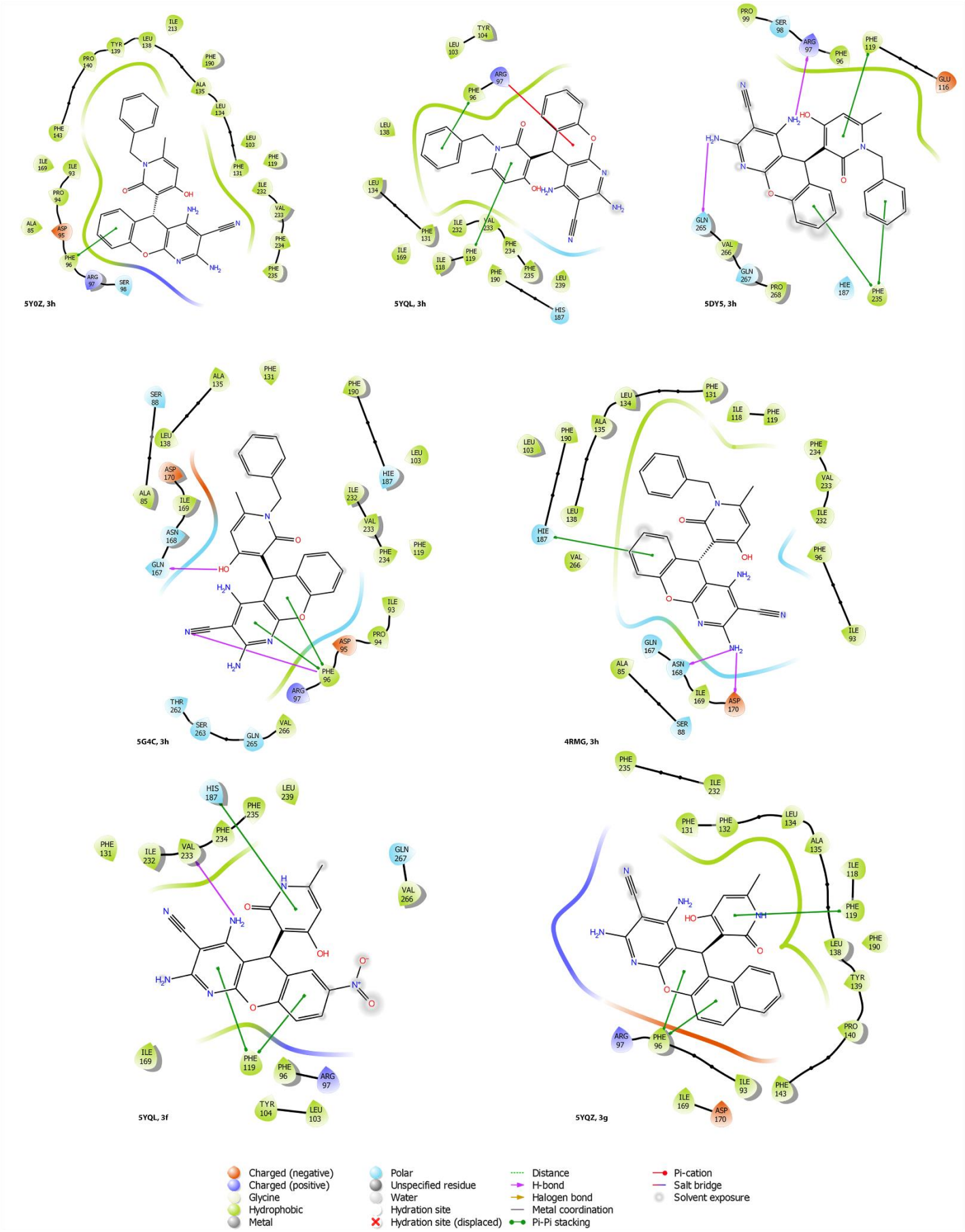


Figure 3. Interaction of **3h** and binding pocket; the best (**3f**) and the worst (**3g**) Emodel ranked interactions

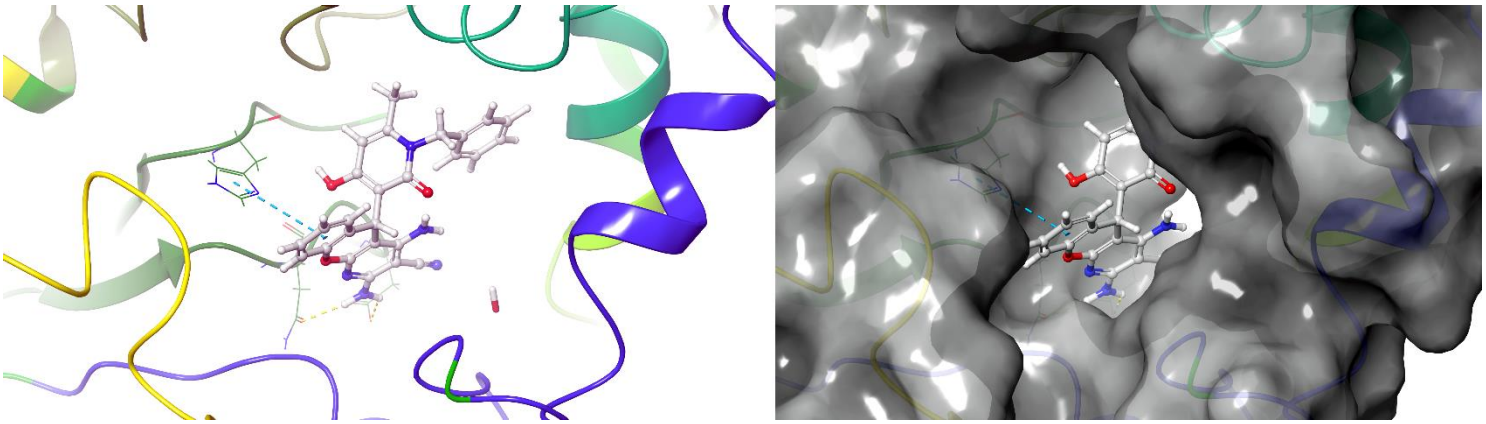


Figure 4. 3D-disposition of compound **3h** into 4RMG binding pocket, helix (left) and surface (right) interpretations.

Conclusions

The efficient and facile pseudo-four-component reaction of salicylaldehydes, malononitrile and hydroxy-4-methylpyridin-2(1*H*)-ones leads to formation of 5-(4-hydroxy-2-oxo-1,2-dihydropyridin-3-yl)-substituted 5*H*-chromeno[2,3-*b*]-pyridines which are promising compounds for different biomedical applications. The procedure utilizes simple equipment, readily available and not expensive starting materials, is easily carried out, and the isolation of final *5H*-chromeno[2,3-*b*]pyridines is not labor intensive.

Molecular docking studies of the synthesized 5*H*-chromeno[2,3-*b*]pyridines were carried out to identify their relationship with SIRT2 binding pocket. The results indicate that (1-benzyl-4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-5*H*-chromeno[2,3-*b*]pyridine has good affinity to SIRT2.

Experimental Section

General. All melting points were measured with a Gallenkamp melting-point apparatus. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 with Bruker Avance II 300 spectrometer at ambient temperature. Chemical-shift values are relative to Me_4Si. In some cases, OH, NH_2 and NH signals were exchanged with D_2O (which is present as an impurity in DMSO-d_6). IR spectra were recorded with a Bruker ALPHA-T FT-IR spectrometer using KBr pellets. Mass-spectra (EI 70 eV) were obtained directly with a Kratos MS-30 spectrometer. High-resolution mass spectra (HRMS) (electrospray ionization, ESI) were measured on a BrukerMicroTOF II instrument.

General procedure for preparation of the functionalized *5H*-chromeno[2,3-*b*]pyridines (**3**)

Salicylaldehyde (**1**) (3 mmol), malononitrile (6 mmol, 0.396 g) and 6-hydroxy-4-methylpyridin-2(1*H*)-one (**2**) (3 mmol) were refluxed in 5 mL of pyridine for 2 h. After the reaction was finished, the reaction mixture was frozen for 15 minutes, then the solid was filtered off, washed with methanol (2 x 2 mL), and dried to isolate pure substituted 5*H*-chromeno[2,3-*b*]pyridine (**3**).

2,4-Diamino-5-(4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**3a**). Yellowish powder; yield 1.00 g, (92%); mp: 317–318°C (decomp.); lit.[30] mp: 315–316°C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.09 (s, 3H, CH_3), 5.33 (s, 1H, CH), 5.56 (s, 1H, CH), 6.25 (s, 2H, NH_2), 6.57 (br s, 2H, NH_2), 6.88-7.02 (m, 3H, 3 CH Ar), 7.10-7.22 (m, 1H, CH Ar), 10.33 (br s, 1H, OH), 11.50 (br s, 1H, NH) ppm.

2,4-Diamino-9-ethoxy-5-(4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**3b**). Yellowish powder; yield 0.90 g, (74%); mp: 298-299 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.38 (t, ^3J 6.8 Hz, 3H, CH_3), 2.08 (s, 3H, CH_3), 4.04 (q, ^3J 6.8 Hz, 2H, OCH_2), 5.31 (s, 1H, CH), 5.54 (s, 1H, CH), 6.28 (s, 2H, NH_2), 6.45-6.61 (m, 3H, NH_2 + CH Ar), 6.78-6.93 (m, 2H, 2 CH Ar), 10.36 (br s, 1H, OH), 11.49 (br s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.8, 18.3, 28.4, 63.8, 70.1, 89.2, 98.3, 99.5, 110.9, 111.5, 116.7, 119.7, 122.7, 124.4, 141.1, 144.3, 145.8, 156.8, 159.1, 164.6, 165.2 ppm; IR (KBr): v = 3351, 3149, 2881, 2203, 1623, 1566, 1478, 1405, 1271, 1097cm^{-1}; MS:*m/z*, (relative intensity, %): 405 [M$^+$] (4), 385 (24), 356 (82), 319 (42), 281 (77), 237 (11), 187 (9), 125 (42), 84 (45), 29 (100). MS (ESI): *m/z* 406.1500 [M + H]$^+$, 428.1324 [M + Na]$^+$, 444.1065 [M + K]$^+$, calcd for $C_{21}H_{19}N_5O_4$: 406.1510 [M + H]$^+$, 428.1329 [M + Na]$^+$, 444.1069 [M + K]$^+$.

2,4-Diamino-5-(4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-8-methoxy-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**3c**)**.** White powder; yield 1.02 g, (87%); mp: 277-278 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.08 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 5.25 (s, 1H, CH), 5.55 (s, 1H, CH), 6.23 (s, 2H, NH_2), 6.50-6.64 (m, 4H, NH_2 + 2 CH Ar), 6.83 (d, ^3J 8.2 Hz, 1H, CH Ar), 10.29 (br s, 1H, OH), 11.48 (br s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 18.3, 27.7, 55.2, 70.2, 89.6, 99.4, 100.4, 109.5, 111.7, 115.9, 116.7, 128.8, 144.2, 152.1, 156.9, 158.4, 159.0, 159.1, 164.6, 165.1 ppm. IR (KBr): v = 3391, 3190, 2696, 2193, 1627, 1605, 1568, 1395, 1294, 1175cm^{-1}; MS:*m/z*, (relative intensity, %): 391 [M$^+$] (3), 371 (14), 267 (100), 237 (8), 224 (25), 195 (2), 153 (1), 125 (51), 84 (53), 16 (89). MS (ESI): *m/z* 392.1346 [M + H]$^+$, 414.1165 [M + Na]$^+$, calcd for $C_{20}H_{17}N_5O_4$: 392.1353 [M + H]$^+$, 414.1173 [M + Na]$^+$.

2,4-Diamino-7-chloro-5-(4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**3d**). Yellowish powder; yield 0.83 g, (70%); mp: 345–346°C (decomp.); lit.[30] mp: 343–344°C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.09 (s, 3H, CH_3), 5.30 (s, 1H, CH), 5.58 (s, 1H, CH), 6.29 (s, 2H, NH_2), 6.59 (br s, 2H, NH_2), 6.89 (d, ^4J 2.2 Hz, 1H, CH Ar), 7.01 (d, ^3J 8.5 Hz, 1H, CH Ar), 7.21 (dd, ^3J 8.5 Hz, ^4J 2.2 Hz, 1H, CH Ar), 10.46 (br s, 1H, OH), 11.54 (br s, 1H, NH) ppm.

2,4-Diamino-5-(4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-7,9-diiodo-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**3e**)**.** Gray powder; yield 0.74 g, (40%); mp: > 330 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.10 (s, 3H, CH_3), 5.28 (s, 1H, CH), 5.58 (s, 1H, CH), 6.47 (s, 2H, NH_2), 6.63 (br s, 2H, NH_2), 7.16 (s, 1H, CH Ar), 7.92 (s, 1H, CH Ar), 10.59 (br s, 1H, OH), 11.56 (br s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 18.4, 28.6, 70.5, 86.1, 87.0, 88.9, 99.3, 111.0, 116.4, 127.3, 136.4, 143.6, 145.0, 151.0, 156.8, 158.7, 159.2, 164.3, 165.3 ppm; IR (KBr): v = 3438, 3353, 3456, 2205, 1626, 1580, 1549, 1446, 1405, 1252 cm^{-1}; MS:*m/z*, (relative intensity, %): 613 [M$^+$] (5), 489 (54), 254 (7), 127 (100), 84 (60), 42 (91). MS (ESI): *m/z* 613.9176 [M + H]$^+$, calcd for $C_{21}H_{19}N_5O_4$: 613.9181 [M + H]$^+$.

2,4-Diamino-5-(4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-7-nitro-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**3f**).Yellow powder; yield 0.73 g, (60%); mp: 310–311°C (decomp.); lit.[30] mp: 311–312°C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.08 (s, 3H, CH_3), 5.39 (s, 1H, CH), 5.58 (s, 1H, CH), 6.42 (s, 2H, NH_2), 6.69 (br s, 2H, NH_2), 7.21 (d, ^3J 8.6 Hz, 1H, CH Ar), 7.73 (d, ^4J 2.2 Hz, 1H, CH Ar), 8.06 (dd, ^3J 8.6 Hz, ^4J 2.2 Hz, 1H, CH Ar), 10.60 (br s, 1H, OH), 11.62 (br s, 1H, NH) ppm.

9,11-Diamino-12-(4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-12*H*-benzo[5,6]chromeno[2,3-*b*]-pyridine-10-carbonitrile (**3g**). Yellowish powder; yield 1.20 g, (97%); mp: 290–291°C (decomp.); lit.[30] mp: 289–290 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.05 (s, 3H, CH_3), 5.50 (s, 1H, CH), 5.82 (s, 1H, CH), 6.31 (s, 2H, NH_2), 6.73 (s, 2H, NH_2), 7.25 (d, ^3J 8.8 Hz, 1H, CH Ar), 7.33-7.50 (m, 2H, 2 CH Ar), 7.77-7.90 (m, 2H, 2 CH Ar), 7.97 (d, ^3J 8.8 Hz, 1H, CH Ar), 10.39 (br s, 1H, OH), 11.56 (br s, 1H, NH) ppm.

2,4-Diamino-5-(1-benzyl-4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**3h**). White powder; yield 1.08 g, (80%); mp: 322–323°C (decomp.), lit.[30] mp: 324–325 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.13 (s, 3H, CH_3), 5.26 (d, ^2J 15.1 Hz, 1H, CH_2), 5.36-5.55 (m, 2H, CH

+ CH_2), 5.71 (s, 1H, CH), 6.26 (s, 2H, NH_2), 6.49 (br s, 2H, NH_2), 6.90-7.03 (m, 3H, 3 CH Ar), 7.10-7.23 (m, 3H, 3 CH Ar), 7.24-7.46 (m, 3H, 3 CH Ar), 10.48 (br s, 1H, OH) ppm.

2,4-Diamino-5-(1-benzyl-4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-8-methoxy-5*H*-chromeno[2,3-*b*]pyridine-3-carbonitrile (**3i**).Yellowish powder; yield 1.27 g, (88%); mp: 300-301 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.12 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 5.24 (d, ^2J 15.9 Hz, 1H, CH_2), 5.37 (s, 1H, CH), 5.45 (d, ^2J 15.9 Hz, 1H, CH_2), 5.72 (s, 1H, CH), 6.23 (s, 2H, NH_2, exchanged with D_2O), 6.48 (br s, 2H, NH_2, exchanged with D_2O), 6.54 (d, ^4J 1.7 Hz, 1H, CH Ar), 6.58 (dd, ^3J 8.8 Hz, ^4J 1.7 Hz, 1H, CH Ar), 6.86 (d, ^3J 8.8 Hz, 1H, CH Ar), 7.07-7.21 (m, 2H, 2 CH Ar), 7.22-7.44 (m, 3H, 3 CH Ar) 10.50 (br s, 1H, OH, exchanged with D_2O) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.8, 29.0, 46.7, 55.3, 89.4, 100.5 (2C), 101.3, 109.8, 111.8, 115.7, 116.7, 126.4 (2C), 127.2, 128.8 (3C), 137.4, 146.1, 152.1, 156.7, 158.6, 159.0, 159.2, 163.5, 164.6 ppm; IR (KBr): v = 3437, 3356, 3125, 2197, 1639, 1568, 1400, 1250, 1175, 1088 cm^{-1}; MS:*m/z*, (relative intensity, %): 481 [M^+] (1), 390 (1), 333 (6), 267 (20), 215 (22), 200 (2), 124 (4), 109 (9), 91 (100), 16 (72). MS (ESI): *m/z* 482.1824 [M + H]$^+$, 504.1647 [M + Na]$^+$, 520.1393 [M + K]$^+$, calcd for $C_{27}H_{23}N_5O_4$: 482.1823 [M + H]$^+$, 504.1642 [M + Na]$^+$, 520.1382 [M + K]$^+$.

2,4-Diamino-5-(1-benzyl-4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-7-chloro-5*H*-chromeno[2,3-*b*]-pyridine-3-carbonitrile (**3j**). Yellowish powder; yield 0.74 g, (51%); mp: 314-315 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.14 (s, 3H, CH_3), 5.21 (d, ^2J 12.5 Hz, 1H, CH_2), 5.33-5.59 (m, 2H, CH + CH_2), 5.76 (s, 1H, CH), 6.30 (s, 2H, NH_2, exchanged with D_2O), 6.49 (br s, 2H, NH_2, exchanged with D_2O), 6.91 (d, ^4J 1.5 Hz, 1H, CH Ar), 7.01 (d, ^3J 8.1 Hz, 1H, CH Ar), 7.06-7.45 (m, 6H, 6 CH Ar), 10.67 (br s, 1H, OH, exchanged with D_2O) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.9, 29.5, 46.7, 88.5, 101.1, 111.2, 116.6, 117.3, 125.8, 126.4 (2C), 126.6, 127.2, 127.4 (2C), 127.5, 128.8 (2C), 137.3, 146.7, 150.3, 156.7, 159.0, 159.1, 163.7, 164.5 ppm; IR (KBr): v = 3463, 3347, 3089, 2204, 1638, 1569, 1475, 1402, 1257, 1094 cm^{-1}; MS:*m/z*, (relative intensity, %): 487 [^{37}Cl, M^+] (1), 485 [^{35}Cl, M^+] (2), 394 (2), 339 (^{37}Cl, 4), 337 (^{35}Cl, 15), 273 (^{37}Cl, 11), 271 (^{35}Cl, 28), 215 (63), 200 (4), 138 (4), 91 (100), 44 (25), 16 (13). MS (ESI): *m/z* 486.1327 [M + H]$^+$, 508.1153 [M + Na]$^+$, 542.0890 [M + K]$^+$, calcd for $C_{26}H_{20}ClN_5O_3$: 486.1327 [M + H]$^+$, 508.1147 [M + Na]$^+$, 542.0886 [M + K]$^+$.

Isolation of 4*H*-chromene-3-carbonitrile (**5**)

Salicylaldehyde (**1a**) (3 mmol, 0.366 g), malononitrile (6 mmol, 0.396 g) and 6-hydroxy-4-methylpyridin-2(1*H*)-one (**2a**) (3 mmol, 0.375 g) were refluxed in 5 mL pyridine for 0.5 h. After the reaction was finished, the solid was filtered off, washed with methanol (2 x 2 mL), and dried to isolate pure 4*H*-chromene-3-carbonitrile (**5**).

2-Amino-4-(4-hydroxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)-4*H*-chromene-3-carbonitrile (**5**). White powder; yield 0.73 g, (82%); mp: 214–215°C (decomp.), lit.[24] mp: 214–216 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.05 (s, 3H, CH_3), 5.09 (s, 1H, CH), 5.62 (s, 1H, CH), 6.52 (s, 2H, NH_2), 6.88-7.15 (m, 4H, 4 CH Ar), 10.22 (br s, 1H, OH), 10.95 (br s, 1H, NH) ppm.

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Supplementary Material

Copies of ^{1}H and ^{13}C NMR spectra of the compounds and additional information on the docking studies are provided in the Supplementary Material file available on the Publisher's website.

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