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Synthesis and evaluation of novel 2-pyridone derivatives as inhibitors of phosphodiesterase3 (PDE3): A target for heart failure and platelet aggregation

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ABSTRACT

Twenty-six 2-pyridone derivatives (**8a–8z**), which are structurally analogous to amrinone and milrinone two important cardiotonic drugs, are synthesized and characterized. The synthesis of 2-pyridone derivatives involves addition, followed by cyclization between Baylis–Hillman acetates (**7a–7k**) and enamino esters or nitriles (**3a–3e**). Thus synthesized pyridones were subjected to PDE3 inhibitory activity, 14 pyridones were found to be hits out of 26 pyridones synthesized and out of 14 hits, there are 5 pyridones found to be lead compounds having excellent PDE3 inhibitory activity. Further we have carried out computational analysis to understand protein/enzyme and 2-pyridone derivative interactions to identify amino acid residues involved in the vicinity of binding and compared with milrinone drug.

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Congestive heart failure (CHF) is a major cause of death in patients with heart disease. Digitalis glycosides (drug that is extracted from the leaves of the foxglove plant) have been used for the treatment of CHF for more than 200 years.¹ However, application of these agents are limited because of their narrow therapeutic window and their propensity that cause life-threatening arrhythmias (arrhythmogenic liability). Thus digitalis has been replaced by a new class of cardiotonic agents named as phosphodiesterase enzyme (PDE) inhibitors. For example amrinone **1** and milrinone **2** (Fig. 1), are 2-oxopyridine derivatives that have been introduced to the clinic for the treatment of CHF in place of digitalis.² These PDE inhibitors exhibit a greater safety profile and improved efficacy on patient survival. Phosphodiesterases are a class of intracellular enzymes responsible for the hydrolysis of cyclic adenosine monophosphate (c-AMP) and cyclic guanosine monophosphate (c-GMP) which are involved in the regulation of important cell functions, such as secretion, contraction, metabolism, and growth.³ On the basis of structure and substrate

specificity, PDE enzymes can be grouped into eleven different families, PDE1 to PDE11.⁴ Each PDE isoenzyme has a conserved C-terminal catalytic domain and unique N-terminal regulatory domain. These isoenzymes are found in different tissues and cells of the humans such as smooth muscle, brain, heart, lung, platelets,

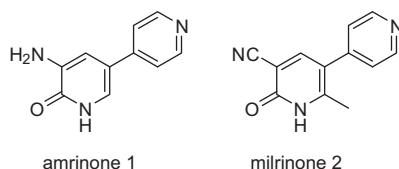


Figure 1. Pyridone based cardiotonic agents.

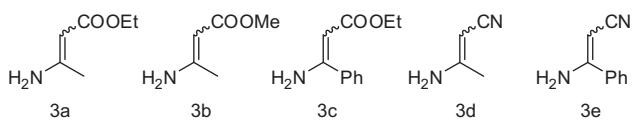
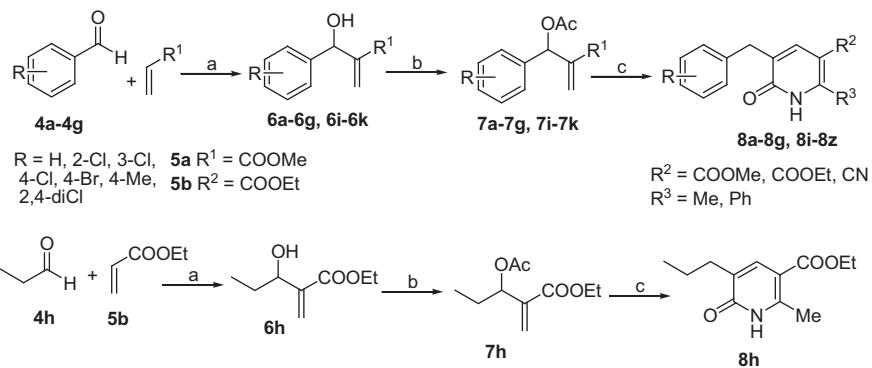


Figure 2. Various enamines used for 2-pyridones synthesis.

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Scheme 1. Reagents and conditions: (a) DABCO, neat, rt; (b) AcCl, pyridine, DCM, DMAP (cat.) 0 °C rt; (c) enamine **3**, NaH, THF, rt.

Table 1
Synthesis of 2-pyridone derivatives **8a–8z** from Baylis–Hillman acetates and enamines

Entry	BH acetate	Enamine	Product	Yield ^a (%)
1		3a		82
2		3a		80
3		3a		78
4		3a		78
5		3a		76
6		3a		77
7		3a		75
8		3a		77
9		3b		78
10		3b		74

(continued on next page)

Table 1 (continued)

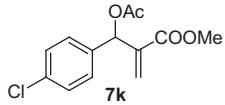
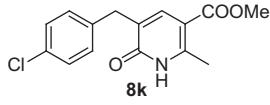
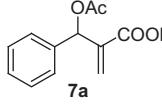
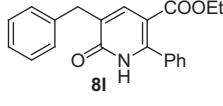
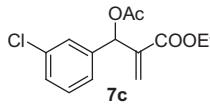
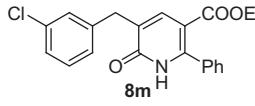
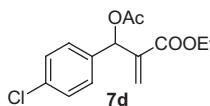
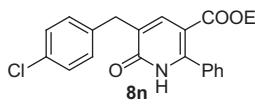
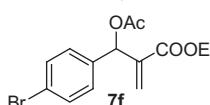
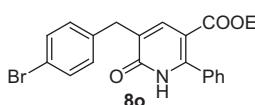
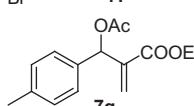
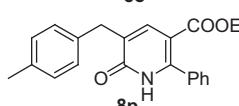
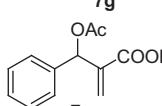
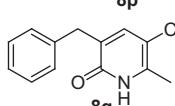
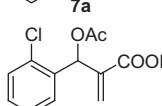
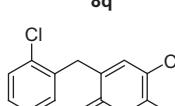
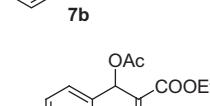
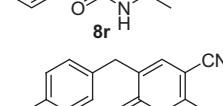
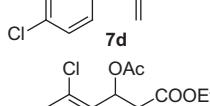
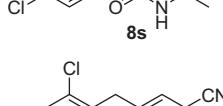
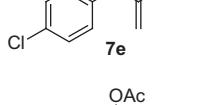
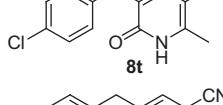
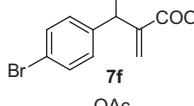
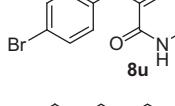
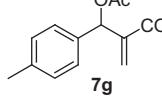
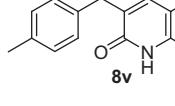
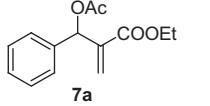
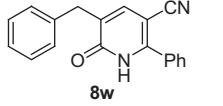
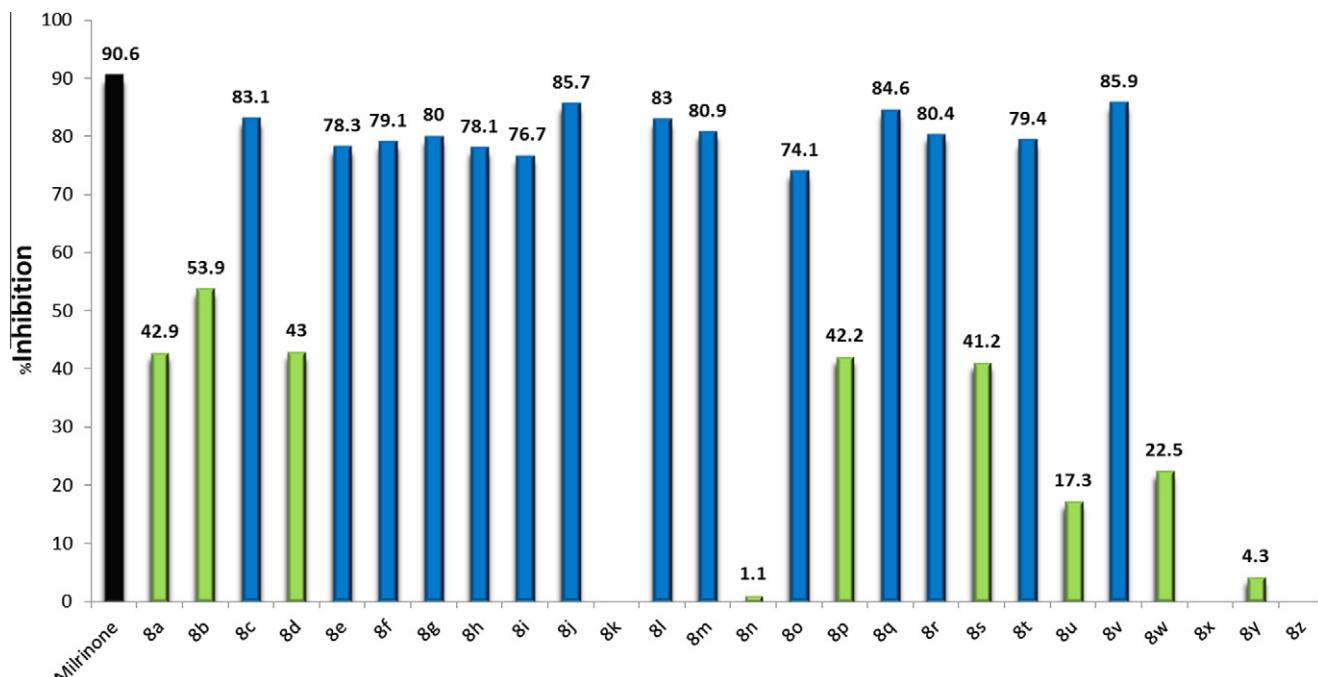
Entry	BH acetate	Enamine	Product	Yield ^a (%)
11		3b		78
12		3c		76
13		3c		78
14		3c		77
15		3c		72
16		3c		69
17		3d		77
18		3d		76
19		3d		74
20		3d		75
21		3d		75
22		3d		65
23		3e		68
24		3e		70

Table 1 (continued)

Entry	BH acetate	Enamine	Product	Yield ^a (%)
25		3e		70
26		3e		67

^a Isolated yields.**Figure 3.** PDE3 inhibitory activity of 2-pyridone derivatives (500 μM in DMSO). Standard Drug: Milrinone (500 μM in DMSO).

lymphocytes etc. and in other species.⁵ PDE3 and PDE4 are well established in cardiovascular diseases⁶ because they are predominantly expressed in heart and platelets to play an important role in controlling heart contraction and platelet aggregation.^{7–9}

Inhibition of PDE¹⁰ brings about various physiological reactions, for example inhibition of PDE3 enhances myocardial contraction, produces vasodilatation, and suppresses platelet aggregation.¹¹ These are the reasons why PDE3 inhibitors can be used to treat heart failure. In addition, the PDE3 isoenzyme is specific for c-AMP and has no effect on c-GMP and calmodulin. Therefore, inhibition of PDE3 isoenzyme in cardiovascular tissues may lead to high levels of c-AMP and consequent inotropic effect. Recent studies revealing that PDE3, PDE4 and PDE5 isoenzymes are over-expressed in cancerous cells compared with normal cells.¹² Thus inhibition of PDE3 together with other PDEs may lead to inhibition of tumor cell growth and angiogenesis.¹² Considering the importance of PDE inhibition agents, there is a need to explore newer drugs or agents. In continuation of our work on the synthesis of new heterocyclic compounds¹³ and their biological applications, herein we report the synthesis of novel 2-pyridone derivatives (**8a–8z**) structurally related to amrinone and milrinone using Baylis–Hillman strategy and their target for PDE3 inhibitors.

Five enamines **3a–3e** (Fig. 2) are prepared in gram quantities according to the published procedures¹⁴ and used them as

intermediates in making 2-pyridones. Benzaldehydes **4a–4g** and propionaldehyde **4h** were selected and treated with methylacrylate **5a** or ethylacrylate **5b** under solvent free Baylis–Hillman (BH) conditions¹⁵ to get corresponding BH-adducts **6a–6k** (Scheme 1). Thus prepared BH-adducts were converted into corresponding BH-acetates **7a–7k** under standard acetylating conditions^{15d} and these acetates served as key intermediates in the synthesis of 2-pyridones. The base, sodium hydride induced reaction between BH acetate and enamine derivative in THF as solvent afforded the targeted 2-pyridones. The complex chemistry of formation of 2-pyridone is discussed earlier.¹⁶ The BH acetates **7a–7k** in combination with enamines **3a–3e** resulted in 26 2-pyridones **8a–8z** (Table 1), out of which 14 are new, in very good yields. The entire synthetic reactions with reagents used are depicted in Scheme 1. Thus synthesized 2-pyridones **8a–8z** characterized very well by spectral means and their purity was ascertained using HPLC. The 26 2-pyridones synthesized differ in their position of groups/appendages attached on the 2-pyridone ring were screened to evaluate their PDE3 inhibitor activity.

The *in vitro* phosphodiesterase3 (PDE3) inhibitory activity of 26 compounds were measured using a Biomol Green™ Quantizyme Assay System (catalogue no. BML-AK800-0001) as described in the literature.¹⁷ The basic principle for this assay is to cleave c-AMP or c-GMP into their respective nucleotide by a cyclic

Table 2
PDE3 inhibitory activity (IC_{50})

S. No	Esters	IC ₅₀ (µM)	S. No	Nitriles	IC ₅₀ (µM)
1	8c	7.49	11	8q	128.3
2	8e	172.13	12	8r	423.2
3	8f	38.5	13	8t	280.7
4	8g	172.5	14	8v	1.68
5	8h	125.1	15	Milrinone	3.3
6	8i	163.5	16	Milrinone	153 ¹⁸
7	8j	2.66	17	Milrinone	12.4 ¹⁹
8	8l	7.3	18	Milrinone	10 ²⁰
9	8m	312.4	19	Milrinone	2 ²¹
10	8o	8.47			

(i) Entry no. 15 is our own result; (ii) entry nos. 16–19 are the reported results with references cited; (iii) standard deviation: all experiments were independently performed three times.

Table 3
Molecular docking scores of selected molecules

Molecule	Dock score	IC ₅₀	Protein–ligand interactions ^a
8c	−12.4	7.49	His29; Asn34; Thr33; Leu99; Phe161
8j	−12.6	2.66	His29; Asn34; Thr33; Phe616; Leu99
8k	−8.1	—	Leu99; Thr97; His29; Asn34; Thr33; Glu55
8l	−9.1	7.3	Asp139; Asp26; His29; Leu99; Thr97; His25; Ile157
8n	−8.0	—	Asn34; Asp98; Lys100; Thr33; Leu99; Phe193
8o	−8.7	8.47	Asn34; Thr33; Leu99; Asp98; Lys100; Phe193
8v	−17.2	1.68	His29; Leu99; Phe161; Phe193
Milrinone	−13.1	3.3	His29; Asn34; Thr33; Leu99; Ile157

^a Amino acid residues in bold represents H-bond interactions and the rest are Van der Waals interactions.

nucleotide phosphodiesterase. The nucleotides, AMP or GMP released is further cleaved into nucleoside and phosphate by the enzyme 5'-nucleotidase. The extent of phosphate thus released is directly proportional to the PDE3 activity. In this assay, the phosphate released by the enzymatic cleavage is quantified using BIO-MOL GREEN reagent in a modified malachite green assay. The resulting green color with λ_{max} at 620 nm is directly proportional to the released phosphate and hence PDE activity. Milrinone, a known PDE3 inhibitor has been used as standard drug for comparison

with the inhibitory activity of synthesized new analogs. The results of all 26 compounds for PDE3 inhibitory activity are presented in Figure 3. The experiments carried out for all test compounds at 500 μ M concentration revealed that 14 2-pyridone derivatives possess PDE3 inhibitory activity as compared to the standard drug, milrinone. The inhibitory concentration at 50% (IC_{50}) PDE3 activity tested for compounds was calculated from dose response curves obtained by plotting the percentage inhibition versus the concentration and are summarized in Table 2. Among all the 26 2-pyridone derivatives, **8v** and **8j** has shown highest inhibition with IC_{50} of 1.68 and 2.66 μ M respectively. On the other hand, esters such as **8c**, **8l** and **8o** exhibited IC_{50} values of 7.49, 7.3 and 8.47 μ M, respectively.

Molecular docking studies were performed for selected compounds in order to get an insight about their binding preferences from molecular perspective. FlexX docking tool,²² which is integrated into Molecular Operating Environment (Chemical Computing Group, Canada) was used for this purpose.²³ In silico docking studies were carried out with a homology model of PDE3A target (pdb code: 1LRC.pdb).^{10a} Results of molecular docking and summary of protein-ligand interactions are shown in Table 3 and Figure 4 respectively. Molecular docking results of five active compounds (**8c**, **8j**, **8l**, **8o** and **8v**) were compared with standard ligand, that is, milrinone. Docking data indicate that the compound **8v** scored more than any other compound which is consistent with experiments. Scores of compounds **8c** and **8j** are comparable with standard ligand and under estimated results were found in case of compounds **8l** and **8o**. To test the reliability of docking model, inactive compounds were docked into the active site of PDE3A and found the least scores. Protein-ligand interactions of compound compounds **8c** and **8j** display favorable H-bond interactions as milrinone that is, pyridone -NH interacts with His29 and carbonyl 'O' of ester interacts with Asn34. Compound **8v** exhibits H-bond interactions with His29 and Leu99. Substituted benzyl group is in close proximity of hydrophobic amino acids. 2-D interactions of **8l** and **8o** show that it interacts with Asn34 and Asp139 respectively. The noticeable common feature in **8l** and **8o** to differ with **8c**, **8j** and **8v** is the presence of phenyl group in place of methyl group. This indirectly infers that presence of phenyl group is not tolerable at 6th position of 2-pyridone. To conclude from the above sections, presence of pyridone ring is essential in milrinone but comparable potency can be obtained by positioning ester and substituted benzyl groups in place of pyridine and cyano group.

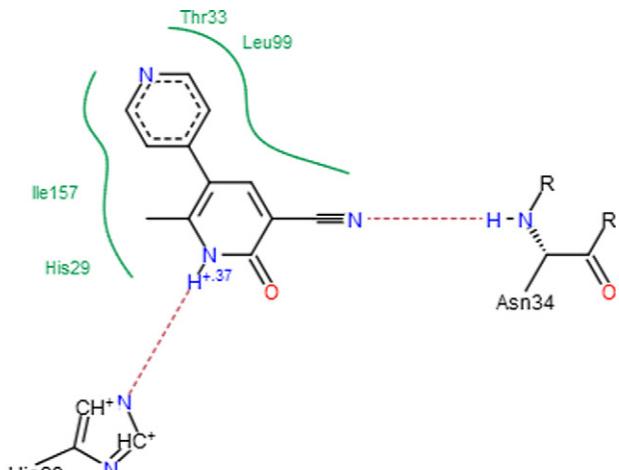
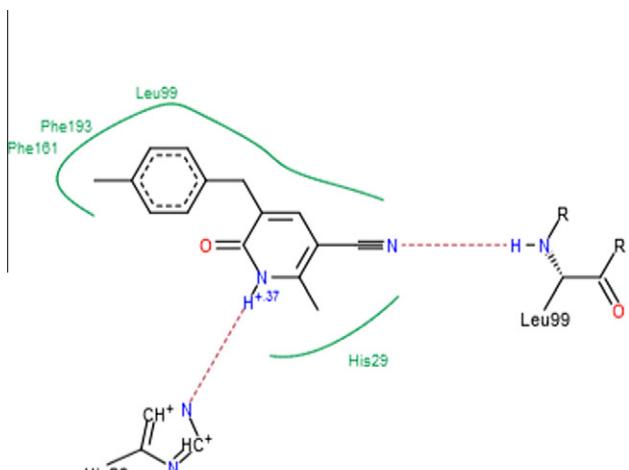


Figure 4. Protein-ligand interactions of **8v** and milrinone as displayed by the best docking orientation for each molecule in the MOE-FlexX integrated docking tool.

that is, 5th and 3rd positions of 2-pyridone respectively. Further it is noticed that the presence of less bulky group at 6th position is tolerable.

Conclusion

Baylis–Hillman acetates (**6a–6k**) and enamines (**3a–3e**) are prepared. The addition of enamine to Baylis–Hillman acetate and followed by cyclization provided 2-pyridones. Twenty-six 2-Pyridone derivatives (**8a–8z**) were generated from the combination of BH acetates and enamines. All the synthesized 2-pyridone derivatives (**8a–8z**) were subjected to PDE3 inhibitory activity. Out of 26 compounds, five 2-pyridone derivatives (**8c, 8j, 8l, 8o** and **8v**) were identified as lead compounds. Computational analysis provided an insight about the molecular interactions between 2-pyridones and PDE3A molecular target. By changing the groups and positions on the 2-pyridone moiety/ring informed that one can achieve PDE3 inhibitory activity and can generate lead compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2012.05.019>.

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