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Synthesis and biological evaluation of novel 5(*H*)-phenanthridin-6-ones, 5(*H*)-phenanthridin-6-one diketo acid, and polycyclic aromatic diketo acid analogs as new HIV-1 integrase inhibitors

Shivaputra Patil,[a] Shantaram Kamath,[a] Tino Sanchez,[b] Nouri Neamati,[b,*] Raymond F. Schinazi[c] and John K. Buolamwini[a,*]

[a]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163, USA*
[b]*Department of Pharmaceutical Sciences, School of Pharmacy, University of Southern California, 1985 Zonal Avenue, Los Angeles, California 90089, USA*
[c]*Emory University School of Medicine/VA Medical Center, Medical Research 151H, 1670 Clairmont Road, Decatur, GA 30033, USA*

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Abstract—A new series of phenanthridinone derivatives, and diketo acid analogs, as well as related phenanthrene and anthracene diketo acids have been synthesized and evaluated as HIV integrase (IN) inhibitors. Several new β-diketo acid analogs with the phenanthridinone scaffold replaced by phenanthrene, anthracene or pyrene exhibited the highest IN inhibitory potency. There is a general selectivity against the integrase strand transfer step. The most potent IN was 2,4-dioxo-4-phenanthren-9-yl-butyric acid (**27f**) with an IC_{50} of 0.38 μM against integrase strand transfer. The phenanthrene diketo acids **27d–f** were more potent (IC_{50} = 2.7–0.38 μM) than the corresponding phenanthridinone diketo acid **16** (IC_{50} = 65 μM), suggesting that the polar amide bridge in the phenanthridinone system decreases inhibitory activity relative to the more lipophilic phenanthrene system. This might have to do with the possible binding of the aryl group of the compounds binding to a lipophilic pocket at the integrase active site as suggested by the docking simulations. Molecular modeling also suggested that effectiveness of chelation of the active site Mg^{2+} contributes to IN inhibitory potency. Finally, some of the potent compounds inhibited HIV-1 replication in human peripheral blood mononuclear cells (PBMC) with EC_{50} down to 8 μM for phenanthrene-3-(2,4-dioxo)butyric acid (**27d**), with a selectivity index of 10 against PBMCs.

1. Introduction

Acquired immunodeficiency syndrome (AIDS), a disease resulting from infection with human immunodeficiency virus (HIV), is one of the world's most serious health problems. It is estimated that approximately 39 million people are living with HIV/AIDS worldwide, with an infection and death rates of around 4 million and 3 million per year, respectively.[1] Three essential enzymes are encoded by the HIV *pol* gene: (1) reverse transcriptase (RT), (2) integrase (IN), and (3) protease (PR)[2] have been the subjects for anti-HIV drug development among others. Drugs targeting RT and PR have been available for over a decade and have shown efficacy particularly when employed in combination.[3–5] However, infection still cannot be eradicated completely with current highly active antiretroviral therapy (HAART) combination treatments, and toxicity and drug resistance are problems as well.[6,7] Thus, there is a need for new inhibitors that could block the virus at other steps of its replication cycle. HIV integrase (IN) is an attractive potential drug target in this regard because it is responsible for incorporation of HIV provirus into the host cell genome, is essential for viral replication, and does not have a direct human counterpart.[8–11] The pertinent catalytic activity of IN involves: (1) the cleavage of a dinucleotide fragment from each end of the proviral

Keywords: HIV integrase inhibitors; Phenanthridinones; Phenanthridinone diketo acid; Polycyclic aromatic diketo acids; Inhibition of HIV replication; Selectivity; Peripheral blood mononuclear cells.
* Corresponding authors. Tel.: +1 901 448 7533; fax: +1 901 448 6828 (J.K.B.); Tel.: +1 323 442 2341; fax: +1 323 442 1390 (N.N.); e-mail addresses: neamati@usc.edu; jbuolamwini@utmem.edu

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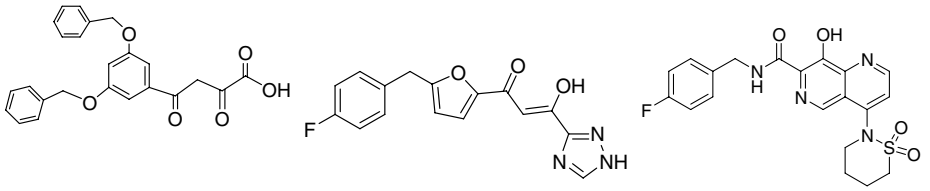
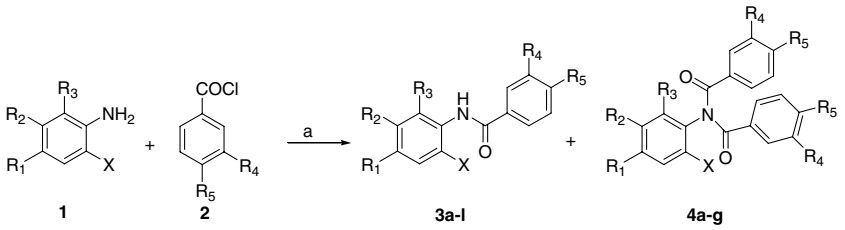
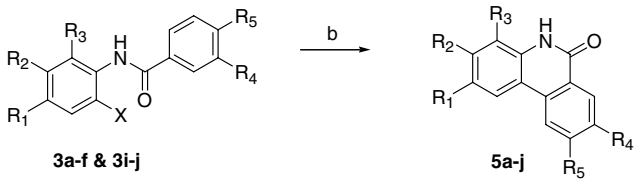


DNA (i.e., 3′-end processing) and (2) insertion of this donor DNA into the host cellular DNA (i.e., strand transfer).[12–14] For almost the past decade and half, many integrase inhibitors with diverse structural features have been identified, designed, synthesized, and screened for the inhibitory activity, and the results show



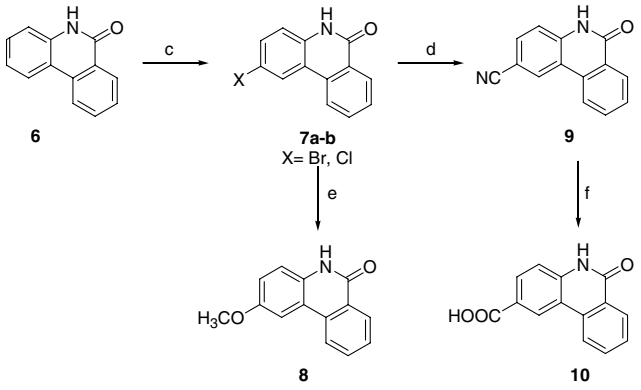
No.	X	R_1	R2	R_3	R_4	R_5
3a	Br	CH_3	H	CH_3	CN	H
3b	Cl	H	H	CH_3	CN	H
3c	Br	CH_3	H	CH_3	H	CN
3d	Br	CH_3	H	CH_3	H	CF3
3e	Cl	H	H	CH_3	H	CN
3f	Cl	H	H	CH_3	H	CF3
3g	Br	CH_3	H	CH_3	NO2	H
3h	Br	CH_3	H	CH_3	H	NO2
3i	Br	CH_3	H	CH_3	H	H
3j	Cl	H	H	CH_3	H	H
3k	Br	CF_3	H	H	H	H
3l	Br	H	$COCH_3$	H	H	H
4a	Br	CH_3	H	CH_3	CN	H
4b	Cl	H	H	CH_3	CN	H
4c	Br	CH_3	H	CH_3	H	CN
4d	Cl	H	H	CH_3	H	CN
4e	Cl	H	H	CH_3	H	CF_3
4f	Br	CH_3	H	CH_3	NO2	H
4g	Br	CH_3	H	CH_3	H	NO_2

Scheme 1. Reagents and condition: (a) Et_3N, CH_2Cl_2, RT.



Comp No.	R_1	R2	R_3	R_4	R_5
5a	CH_3	H	CH_3	CN	H
5b	H	H	CH_3	CN	H
5c	CH_3	H	CH_3	H	CN
5d	CH_3	H	CH_3	H	CF_3
5e	H	H	CH_3	H	CN
5f	H	H	CH_3	H	CF_3
5g	CH_3	H	CH_3	H	H
5h	H	H	CH_3	H	H
5i	CF_3	H	H	H	H
5j	H	$COCH_3$	H	H	H

Scheme 2. Reagents and condition: (b) $Pd(OAc)_2$, Na_2CO_3, DMA, 165 °C.



Scheme 3. Reagents and conditions: (c) NBS/NCS, DMF; (d) CuCN, DMA, 150–155 °C; (e) CuI, NaOMe, DMF, reflux; (f) NaOH, EtOH, reflux.

that effective inhibition is possible.[15–20] Compounds incorporating a β-diketo acid (DKA) moiety as present in L-706,908 or bioisosteric analogs thereof as depicted by the diketo triazole in S-3160 and the hydroxynaphthyridine formyl moiety in L-870,810 have yielded clinical integrase inhibitor drug candidates.[21]

Integrase inhibitors can reduce viral load in patients; at the recent 13th retrovirus conference, Merck and Gilead reported positive phase II clinical trial results, respectively, on new integrase inhibitors; thus validating this enzyme as a bona fide AIDS therapeutic target.[22]

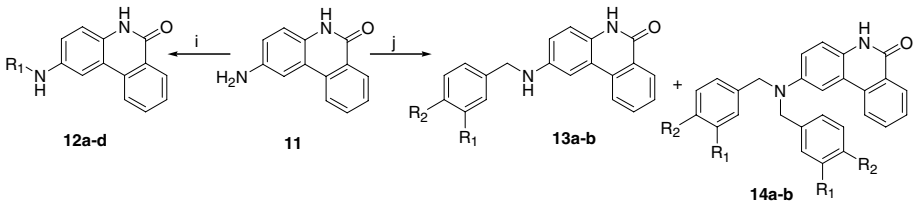
In our search for new integrase inhibitors, we have discovered a series of new substituted phenanthridinones and analogous compounds that show inhibitory activity against the IN catalytic core. The coupling of the phenanthridinone and their biphenyl open ring mimics with the known IN inhibitory activity conferring diketo acid moiety enhanced IN inhibitory activity. Further, converting the phenanthridinone heterocyclic ring system to the more lipophilic phenanthrene or anthracene systems remarkably elevated IN inhibitory potency. Some of the phenanthrene compounds also inhibited HIV-1 replication in cell culture. We report herein the synthesis and biological testing of these diverse new IN inhibitors, as well as results of molecular modeling conducted to explore the binding modes of the potent compounds in the integrase active site.

2. Results and discussion

2.1. Chemistry

Incorporation of specific substituents on the 5*H*-phenanthridin-6-one ring system was achieved by using several synthetic methods (Schemes 1–5). Among these approaches the most convenient method was palladium-catalyzed cyclization of substituted benzamides **3a**–**l** using Na_2CO_3 in dry dimethylformamide at 165 °C yielding the substituted 5*H*-phenanthridin-6-ones **5a**–**j**.[23,24] Substituted benzamides **3a**–**l** were prepared by reacting various 2-haloanilines **1** with substituted benzoyl chlorides **2** in the presence of Et_3N in anhydrous methylene chloride.[25,26] N,N-disubstituted benzamide derivatives **4a**–**g** (2–5%) observed as side products in case of electron-withdrawing groups were present on benzoyl chlorides **2** (Schemes 1 and 2).

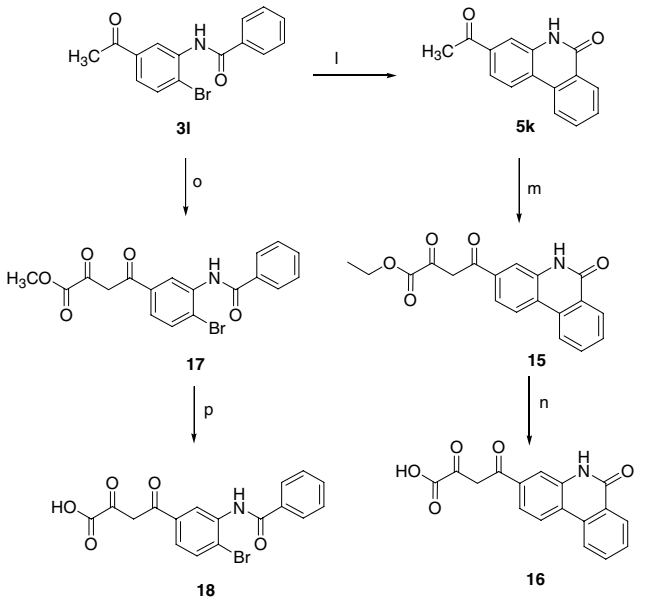
In Scheme 3, 2-bromo-5*H*-phenanthridin-6-ones **7a** and **b** were prepared according to a reported procedure[27] in good yields (70%). 2-Methoxy-5*H*-phenanthridin-6-one **8** was synthesized by refluxing 2-bromo-5*H*-phenanthridin-6-one **7a** with cuprous iodide and sodium methoxide in dry DMF.[28] Compound **7a** was treated with cuprous cyanide in DMA at 150–155 °C to yield the 2-cyano-5*H*-phenanthridin-6-one **9**.[29] Compound **9** was refluxed in ethanolic NaOH to obtain 6-oxo-5,6-dihydrophenanthridine-2-carboxylic acid **10**. A series of N-substituted-2-amino-5*H*-phenanthridin-6-ones, **12a**–**d**, were readily synthesized by stirring 2-amino-5*H*-phenanthridin-6-one, **11**, with appropriate acid chlorides (or ethyl iodide in the case of **12a**) and Et_3N in dry DMF. Compounds **13a** and **b** and **14a** and **b** were prepared by coupling 2-amino-5*H*-phenanthridin-6-one **11** with 3- or 4-bromomethyl benzonitriles by the following three methods. In method A, 2-amino-5*H*-phenanthridin-6-one **11** was stirred with 3- or 4-bromomethyl benzonitriles and Et_3N in dry DMF to yield exclusively **13a**–**b**. Whereas methods B and C, which employed pyridine and K_2CO_3 as bases, respectively, gave **13a** and **b** as ma-



Comp No.	R_1
12a	C_2H_5
12b	$COCH_3$
12c	COC_6H_5
12d	$SO_2C_6H_5$

Comp No.	R_1	R2
13a, 14a	CN	H
13b, 14b	H	CN

Scheme 4. Reagents and conditions: (i) Acid chlorides (or ethyl iodide in the case of **12a**), Et_3N, DMF, RT; (j) 3- or 4-bromomethyl-benzonitrile, K_2CO_3, EtOH, reflux or 3- or 4-bromomethyl-benzonitrile, Et_3N, DMF, RT or 3- or 4-bromomethyl-benzonitrile, pyridine, DMF.

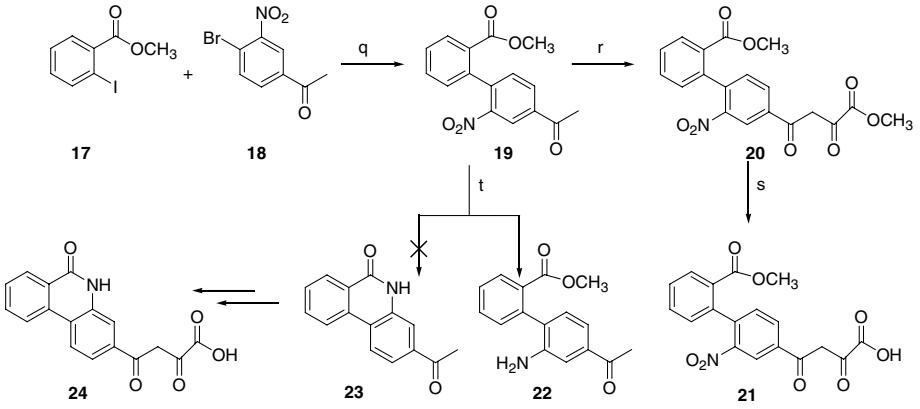


Scheme 5. Reagents and conditions: (l) $Pd(OAc)_2$, Na_2CO_3, DMA, 165 °C; (m) diethyl oxalate, KtOBu, dioxane, reflux; (n) NaOH, methanol; (o) diethyl oxalate, NaOMe, methanol; (p) NaOH, methanol.

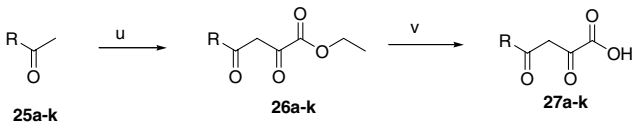
jor products (60%), and **14a** and **b** as minor products (5–6%).

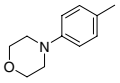
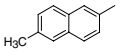
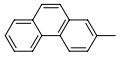
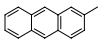
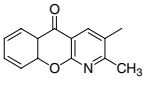
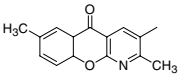
Scheme 5 shows the synthesis of diketo acids **16** and **18**. Treatment of **3l** and **5k** with diethyl oxalate and sodium methoxide provided the 2,4-dioxo-4-(6-oxo-5,6-dihydrophenanthridin-3-yl)-butyric acid ethyl ester **15** and 4-(3-benzoylamino-4-bromo-phenyl)-2,4-dioxobutyric acid methyl ester **17**, which were then hydrolyzed to 2,4-dioxo-4-(6-oxo-5,6-dihydrophenanthridin-3-yl)-butyric acid **16** and 4-(3-benzoylamino-4-bromo-phenyl)-2,4-dioxo-butyric acid **18**, respectively, using methanolic NaOH.

Biphenyl diketo acid **21** was synthesized as depicted in synthetic Scheme 6. Ullmann coupling of 2-iodobenzoic acid methyl ester **17** with 1-(4-bromo-3-nitro-phenyl)ethanone **18** gave the 4′-acetyl-2′-nitrobiphenyl-2-carboxylic acid methyl ester **19**. Reduction of compound **19** with Raney nickel and hydrazine hydrate was intended to yield the cyclic compound 3-acetyl-5*H*-phenanthridin-6-one **23**, but surprisingly we obtained 4′-acetyl-2′-aminobiphenyl-2-carboxylic acid methyl ester **22**.[31] Compound **19** was treated with diethyl oxalate in sodium methoxide to obtain diketoester **20**, which was



Scheme 6. Reagents and conditions: (q) Cu powder, 180–200 °C; (r) diethyl oxolate, NaOMe, MeOH; (s) NaOH, methanol; (t) Raney nickel NH_2NH_2, MeOH.



Compound No.	R
27a	
27b	
27c	
27d	
27e	
27f	
27g	
27h	
27i	
27j	
27k	

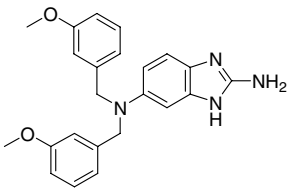
Scheme 7. Reagents and conditions: (u) diethyl oxalate, NaOMe, MeOH; (v) NaOH, methanol.

further selectively hydrolyzed to 4′-(3-carboxy-3-oxo-propionyl)-2′-nitrobiphenyl-2-carboxylic acid methyl ester **21** by methanolic NaOH. Scheme 7 shows the preparation of polycyclic aromatic hydrocarbon diketo acids. Acetyl derivatives **25a**–**k** were treated with diethyl oxalate to obtain the corresponding diketo esters **26a**–**k**. These esters were hydrolyzed directly without further purification to obtain the desired diketo acids **27a**–**k** in 40–90% yields (Table 1).

2.2. HIV-1 integrase inhibition structure–activity relationship

Electron withdrawing groups like CN and CF_3 at the 9-position of the phenanthridinone scaffold (see **5e** and **5f**) along with a substituent at the 4-position conferred moderate inhibitory activity, whereas the presence of an electron withdrawing group such as CN at the 8-position (**5b**) abolished activity. Introduction of the electron donating methyl group at the 2-position as in compound **5a** improved inhibitory activity against both integration steps with IC_{50} values of 300 and 333 μM against 3′-end processing (cleavage) and strand transfer steps, respectively. These observations imply that substitution at the phenanthridinone 2-position is important for inhibitory activity. In other examples, the presence of halogens like Br and Cl at the 2-position (**7a** and **7b**) conferred moderate inhibitory activity, whereas more hydrophilic groups like CN and COOH at the same position produced only low inhibitory activity compounds (**9** and **10**), but provided compounds with selectivity toward strand transfer integration step. Electron-donating groups like NH_2 at the 2-position (compound **11**) improved the inhibitory activity against both 3′-end processing (206 μM) and strand transfer (267 μM) reactions. The NH_2 group might also be involved in hydrogen bonding at the binding site. Compound **14b**, which had a bulky *N*,*N*-bis-(4-cyanobenzyl) substituent, selectively inhibited strand transfer quite well (IC_{50} 62 ± 11 μM) and was the best in phenanthridinone series, whereas its regioisomer, compound **14a** with *N*,*N*-bis-(3-cyanobenzyl) substituents, did not show good inhibitory activity. This indicates that the 2-position tolerates bulky substituents. The fact that the *para*-CN substituted compound (**14b**) exhibited higher potency than the *meta*-CN substituted compound (**14b**) could mean that there is a positive and/or hydrogen-bond acceptor site that is more accessible to the *para*-placed CN than the *meta*-placed CN group. The strand transfer inhibitory selectivity of the more potent compounds in this phenanthridinone series puts them in the same category of IN inhibitory compounds as the diketo acid inhibitors.[30] This observation prompted us to try to augment this moderate strand transfer selectivity by introducing a diketo acid moiety onto the phenanthridinone scaffold to create a hybrid pharmacophore. It is important to note that, to date, one of the most promising classes of integrase inhibitors are the agents with DKA or bioisosteric substitutions thereof[31,32], therefore we went ahead and appended the β-diketo acid moiety to the phenanthridinone. This combination, as exemplified by the first compound synthesized for that purpose, 2,4-dioxo-4-(6-oxo-5,6-dihydro-phenanthridin-3-yl)-butyric acid, **16**, indeed led to a moderate increase in inhibitory activity and selectivity against the strand transfer reaction, relative to the non-DKA-substituted phenanthridinones, thus supporting our hypothesis. It is also interesting that the bis-cyanophenylmethylamine substituted compound, **14b**, the most potent phenanthridinone compound without a DKA moiety, has about the same potency and selectivity as the DKA-substituted phenanthridinone, **16**. The bis-N,N-substituted moiety in **14b** is reminiscent of a similar moiety, bis-methoxyphenylmethylamine, found in IN inhibitory benzimidazole derivatives represented by compound **28**.[21]

For the acquisition of more SAR data, we next turned our attention to examining the following: (1) opening of the central lactam ring of the phenanthridinone ring



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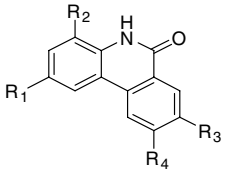
system to afford benzylphenyl ketones and biphenyl compounds as mimics of ring open phenanthridinone analogs, (2) changing one of the phenyl rings of the resulting biphenyl compounds to a reduced heterocycle, and (3) bioisosteric replacement of the phenanthridinone ring system in DKA compound (**16**) with a phenanthrene, anthracene, other polycyclic aromatic systems or heteroaromatic systems like azaxanthone, to obtain diverse aromatic DKAs. The results of these structural modifications turned out to be very interesting with regard to SAR as follows: (1) the benzylphenyl ketone DKAs (**17** and **18**) were practically inactive, whereas the biphenyl-β-diketo ester, **20**, and its acid hydrolysis product **21** displayed better inhibitory activity against strand transfer with IC_{50} values of 27 and 7 μM, respectively (Table 2), than the phenanthridinone DKA, indicating that opening of the central lactam ring of phenanthridinone scaffold to a biphenyl system can lead to enhanced inhibitory activity, (2) the morpholinyl phenyl compound, **27a**, also had improved strand transfer inhibitory activity (IC_{50} = 23 μM, Table 2), and (3) replacement of the phenanthridinone system with the more hydrophobic phenanthrene ring system, as well as other polycarbocyclic aromatics such as anthracene and pyrene, led to a marked increase in IN inhibitory activity, with strand transfer inhibitory activity reaching submicromolar levels. For example, compounds **27f** and **27i** exhibited IC_{50} of 0.38 and 0.53 μM, respectively (Ta-

Table 1. Inhibition of HIV-1 Integrase catalytic activities



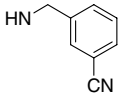
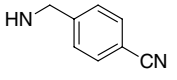
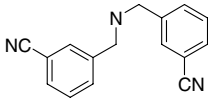
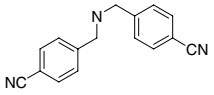
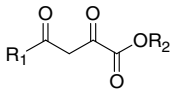
Compound	R_1	R_2	R_3	R_4	3′-end processing IC_{50} (μM)	ST IC_{50} (μM)
5a	CH_3	CH_3	CN	H	300	333
5b	H	CH_3	CN	H	>500	>500
5c	CH_3	CH_3	H	CN	>500	>500
5d	CH_3	CH_3	H	CF_3	>500	>500
5e	H	CH_3	H	CN	>500	500
5f	H	CH_3	H	CF_3	449 ± 99	109 ± 41
5g	CH_3	CH_3	H	H	1000	>1000
5h	H	CH_3	H	H	>1000	>1000
5i	CF_3	H	H	H	>1000	900
7a	Br	H	H	H	225 ± 48	78 ± 37
7b	Cl	H	H	H	500	100
8	OCH_3	H	H	H	>500	500
9	CN	H	H	H	>500	400
10	COOH	H	H	H	>500	203
11	NH_2	H	H	H	206 ± 110	267 ± 153
12a	NHC_2H_5	H	H	H	>500	>500
12b	NH–$COCH_3$	H	H	H	>500	500
12c	NH–COPh				>500	>500
12d	NH–SO_2Ph	H	H	H	>500	415
13a		H	H	H	>500	>500
13b		H	H	H	>500	>500
14a		H	H	H	>500	>500
14b		H	H	H	500	62 ± 11
S1360 (standard)	N/A	N/A	N/A	N/A	11.0 ± 2	0.6 ± 0.1

Table 2. Inhibition of HIV-1 integrase catalytic activities of diketo acid analogs



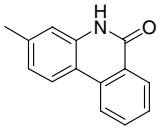
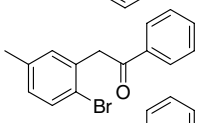
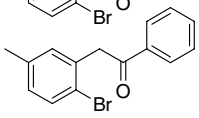
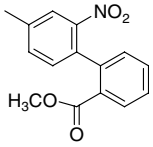
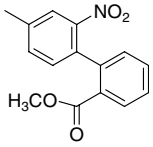
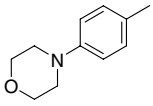
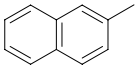
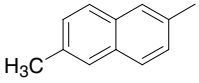
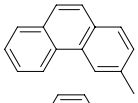
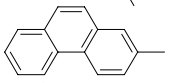
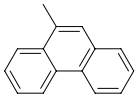
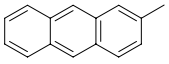
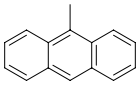
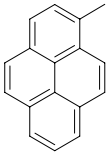
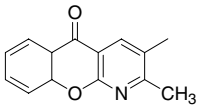
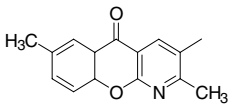
Compound	R_1	R_2	3′-end processing IC_{50} (μM)	ST IC_{50} (μM)
16		H	400 ± 10	65 ± 10
17		CH_3	>500	>500
18		H	>500	>500
20		CH_3	102 ± 3	27 ± 15
21		H	26 ± 10	7 ± 1
27a		H	>100	23 ± 9
27b		H	54 ± 16	7 ± 3
27c		H	11 ± 7	3 ± 2
27d		H	25 ± 7	2.7 ± 2
27e		H	23 ± 6	4.8 ± 2
27f		H	9 ± 2	0.38 ± 0.11
27g		H	32 ± 9	13 ± 4
27h		H	54 ± 10	1.7
27i		H	7 ± 2	0.53 ± 0.25

Table 2 (*continued*)

Compound	R_1	R_2	3′-end processing IC_{50} (μM)	ST IC_{50} (μM)
27j		H	>100	35 ± 15
27k		H	92 ± 5	16 ± 8
S1360 (standard)	N/A	N/A	11.0 ± 2	0.6 ± 0.1

ble 2). These compounds turned out to be the most potent and selective compounds synthesized in our study, with **27f** and **27i** being 13- and 24-fold selective toward strand transfer relative to 3′-end processing, respectively. The azaxanthone DKAs **27j** and **27k** also showed moderate improvement in inhibitory activity over the phenanthridinone DKA compound **16**. Thus, the preference of a very hydrophobic polycyclic aromatic moiety of these new aryl DKAs is clearly indicated by the results.

The concern with these flat polycyclic aromatics is that they may be DNA intercalators, and possibly interfering with donor and/or target in the integration step. This appears not to be the case because we did not observe DNA retardation in our gel electrophoresis experiments. An interesting observation, however, is that the position of diketo acid substitution on these polycyclic aromatic compounds is very important in the SAR. Substitutions that have the diketo acid long axis aligned with the long axis of the polycyclic compound (as in **27d**, **27e**, and **27g**) are less potent than the isomers in which the diketo acid long axis is perpendicular or almost perpendicular to the long axis of the polycyclic system (as in **27f** and **27h**). This positional effect seems to be contrary to a DNA intercalation mechanism, as the former compounds (i.e., **27d**, **27e**, and **27g**) should probably more easily intercalate than the latter compounds (i.e., **27f** and **27h**), and thus be more potent as well, but that was not the case.

2.3. Inhibition of HIV replication in culture

To assess the potential utility of the new compounds as anti-HIV agents, we tested selected potent compounds for the ability to inhibit HIV replication in primary human peripheral blood mononuclear cells (PBMC). The toxicity of the compounds was also evaluated using three cell types: PBMC, human CEM lymphoblastic leukemia cells, and African green monkey Vero cells. The results are presented in Table 3 and indicate that some of the compounds exhibit selective inhibition of HIV replication in cell culture. The most potent compound, **27d**, exhibited an EC_{50} of 8.0 μM in these initial results, which is reasonable anti-viral activity when compared to the anti-viral activity of reported diketo acid IN inhibitors. For example, compound L-706,908 (vide supra) has been shown to inhibit HIV replication with an EC_{50} of 5.7 ± 4.7.[33] To put these results in perspective with regard to AZT's activity, in the same assay, the known diketo acid IN inhibitor L-706,908[21] exhibited an EC_{50} of 5.7 ± 4.7 μM, and AZT exhibited an EC_{50} of 0.001 μM.[33] Thus, the large difference between the EC_{50} values of AZT and compound **27d** is not unusual for DKAs. Moreover, this cell culture HIV inhibitory activity of **27d** is comparable to that of 5*H*-pyrano[2,3-*d*:-6,5-*d*′]dipyrimidines reported recently such as V-165 (EC_{50} of 14.7 μM).[34] Interestingly, compound **27d** also exhibited a reasonable therapeutic index, with a selectivity index of 10 with reference to PBMC, the cell system used for the anti-viral assays. One aspect of the biological activity that is notable is that, the activity was not correlated with IN inhibitory activity. Compound **27f** is the most potent IN inhibitor among the new compounds, but it exhibited a higher EC_{50} (83.7 μM) than compound **27d** (EC_{50} = 8 μM), which exhibited the highest activity against HIV replication in PBMC. Compound **27e** was the second best inhibitor of HIV replication, whereas compound **27h** was virtually inactive.

Table 3. Anti-HIV activity of selected novel potent diketo acids

Compound	Anti-HIV-1 activity in PBMCs[a]		Slope	R	Cytotoxicity (IC_{50}, μM) in		
	EC_{50}(μM)[b]	EC_{90} (μM)[b]			PBMCs	CEM	Vero
AZT (control)	0.00073	0.019	0.68 ± 0.06	0.99	>100	14.3	50.0
27e	26.6	>100	1.5 ± 0.26	0.98	>100	16.2	10.8
27f	83.7	>100	1.1 ± 0.37	1.0	83.5	73.1	>100
27d	8.0	72.8	1.0 ± 0.19	0.96	81.0	56.4	>100
27h	>100	>100			>100	>100	>100
L-706,908	5.7 ± 4.7[c]	NA	NA	NA	NA	NA	NA

[a] Human peripheral blood mononuclear cells (PBMCs) were infected with HIV-1/LAI.
[b] EC_{50} and EC_{90} are the effective concentrations inhibiting 50% and 90% HIV replication, respectively.
[c] In the human leukemic T-cell line MOLT-4; in that assay, the EC_{50} of AZT was 0.001 μM.[34]

Interestingly, the two least potent inhibitors of HIV replication, among compounds tested, were compounds **27f** and **27h** in which the diketo acid long axis is perpendicular or almost perpendicular to the long axis of the polycyclic ring system, whereas the more potent HIV inhibitory compounds **27d** and **27e** have the diketo acid long axis aligned with the long axis of the polycyclic system. However, these examples are too few for any conclusions to be drawn. The differences in anti-viral activity could also be the result of solubility or cell permeability differences among the compounds. These factors will be examined in future testing.

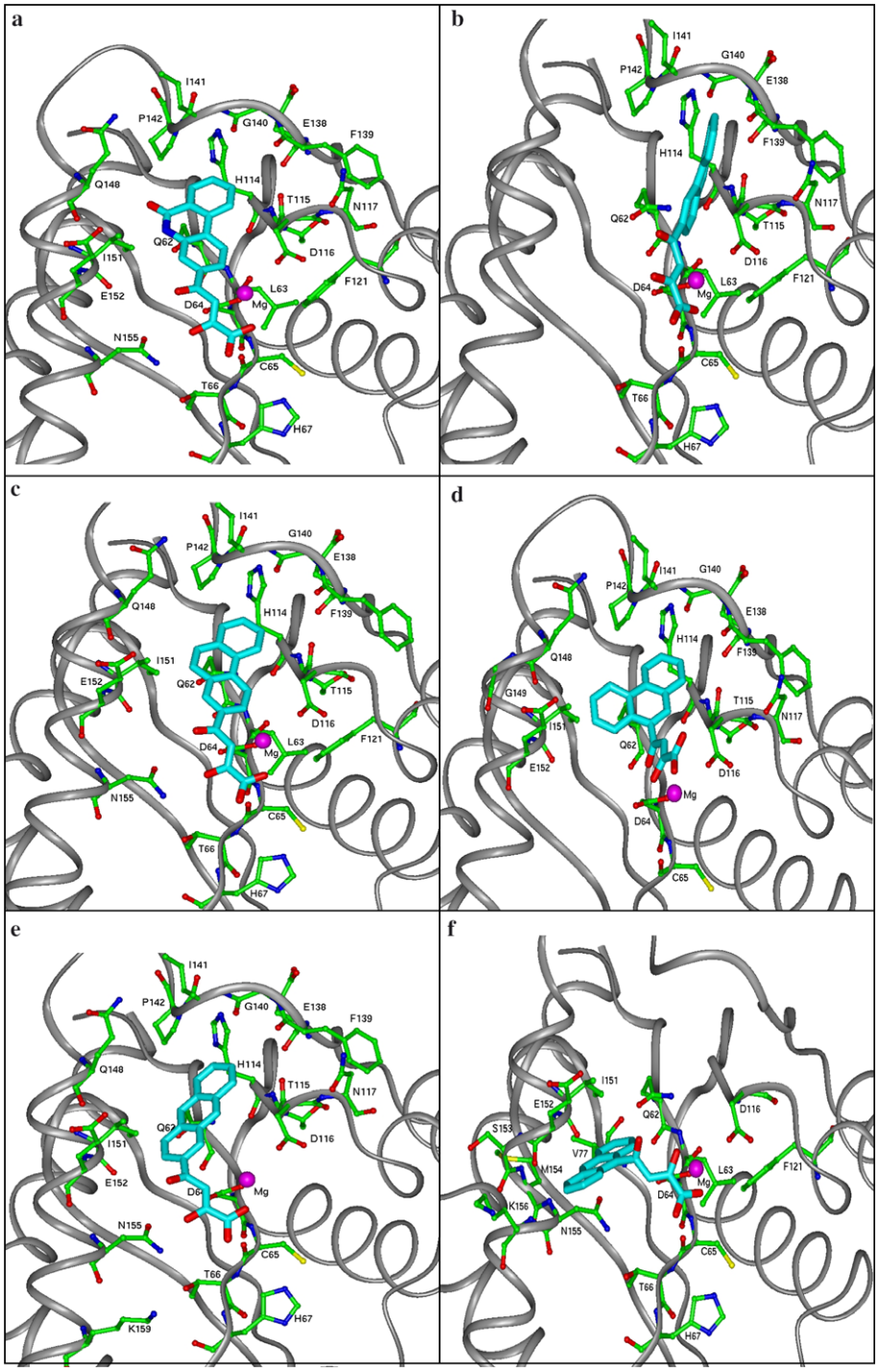


Figure 1. Docking of phenanthridinone diketo acid and polycyclic aromatic diketo acids into HIV integrase catalytic site. Binding modes depicted are: (a) parent phenanthridinone diketo acid, compound **16**, (b) phenanthrene-3-β-diketo acid, compound **27d**, (c) phenanthrene-2-β-diketo acid, compound **27e**, (d) phenanthrene-9-β-diketo acid, compound **27f**, (e) anthracene-2-β-diketo acid, compound **27g**, and (f) anthracene-9-β-diketo acid, compound **27h**. The docked compounds are shown as colored cyan. The protein is represented by gray ribbons, and, active site amino acid residues within 5 Å radius of each docked compound are displayed in green. The Mg^{2+} ion is colored magenta. Other atoms are colored as follows: O, red; N, blue; S, yellow. Hydrogen atoms have been omitted to reduce crowding.

2.4. Molecular modeling

To explore the binding modes of the new compounds at the HIV integrase catalytic site, we conducted binding simulations using the GOLD molecular docking program[35] for the phenanthridinone diketo acid and the potent polycyclic diketo acids as presented in Figure 1. We used the crystal structure of the integrase catalytic core in which the flexible catalytic loop has been resolved, PDB ID 1BIS,[36] to perform docking simulations. We docked the phenanthridinone diketo acid compound **16**, and several potent phenanthrene and anthracene diketo acids. As can be seen in the panels in Figure 1, the aryl part of compounds binds into the hydrophobic pocket involving the flexible catalytic loop region, while the diketo acid moiety is extended to interact with the Mg^{2+} ion as one would expect since it is generally believed that β-diketo acid moiety chelates Mg,[37,38] the most probable divalent cation in the active site of IN in vivo, although this has not yet been proven. The degree of interaction between the DKA moiety and the Mg ion appears to correlate with potency, with the more potent compounds, such as compounds **27f** (Fig. 1d) and **27h** (Fig. 1f), having two oxygen atoms, the enolized oxygen, and one of the carboxylate oxygens interacting with the Mg ion. This is not the case in their less potent counterparts, compounds **16**, **27d**, **27e**, and **27g**, shown in Figure 1a–c and e, respectively, where only one oxygen atom, a carboxylate oxygen, is interacting with the Mg ion. This implies that the more potent compounds are able to dock in a pose (configuration) that allows more effective chelation of the active site Mg^{2+} ion. The favorable orientation of the diketo acid moiety in the compounds such that there was more effective chelation of the active site Mg^{2+} is also suggested by the docking results to influence IN inhibitory activity, with the compounds which involved two oxygen atoms of the diketo acid in interaction with the Mg ion being more potent than those that involved only one oxygen in this interaction. In addition to binding to the active site Mg^{2+} ion to different extents, the docking results suggest these inhibitors also interacted with integrase active site amino acid residues to varying degrees (amino acid residues within 5 Å radius of each docked compound are displayed in Fig. 1) as described in the following. Compound **16**, which has the phenanthridinone moiety, interacts extensively with Asp64, Asp116, and Pro142, and substantially with His114 and Thr115, and to a limited extent with Gln62 and Ile151. Compound **27d** interacts extensively with Asp116, and also interacts substantially with Thr115, Gly140, and Ile141. It also interacts with Phe139. Compound **27e** interacted extensively with Gln62, His114, and Pro142, substantially with Thr115 and Ile151, and limited interaction with Asp116. Compound **27f** interacted extensively with His114, Asp116, Pro142, substantial interaction with Ile151, and limited interaction with Gln62 and Thr115. Compound **27g** interacted extensively with Asp116, substantially with Gln62, Phe139, and Pro142, and limited interaction with Gly140. Compound **27h** interacted extensively with Asp64 and Asn155, and substantially with Ile151 and Glu152. Hydrophobic residues in the catalytic loop like Gly140, Pro142, and Ile151 interact with the lipophilic polycyclic aromatic rings, while hydrophilic residues within the vicinity of the Mg^{2+} ion such as Asp64, Asp116, and Asn155 interacted with the polar diketo acid moiety region of the molecules. This might have to do with the possible binding of the aryl group of the compounds binding to a lipophilic region of the integrase active site as suggested by the docking simulations.

3. Conclusion

In summary, we have successfully synthesized and evaluated the HIV-1 integrase inhibitory activities of series of new phenanthridinones, diketo acid substituted phenanthridinones, and related compounds. Generally, the compounds were selective against IN strand transfer step as compared to the 3′-end processing step of integration, similar to the much studied DKA class of IN inhibitors.[21] Introduction of the DKA functionality into the phenanthridinone system resulted in enhancement of inhibitory activity, and replacement of the phenanthridinone ring system with more lipophilic polycyclic aromatic bioisosteres dramatically increased IN inhibitory potency. Molecular docking simulations identified a possible binding region in the IN catalytic site for these novel potent polycyclic DKAs that could further drug design. More importantly, some of the new potent phenanthrene diketo acid analogs also inhibited HIV replication in cell culture, with good selective toxicity. To the best of our knowledge, this is the first report of potent phenanthrene, anthracene, and pyrene DKA IN inhibitors, and the fact that they possess anti-HIV activity in cell culture with low toxicity makes them a new lead series for further exploration in attempts to discover novel DKA IN inhibitors. Thus, we have expanded the DKA IN inhibitor armentarium, and this is timely as clinical results are beginning to show promise for the use of DKAs as anti-HIV therapeutics.[22]

4. Experimental

All the reagents and solvents were purchased from Aldrich and used without further purification. Progress of all reactions were monitored by TLC on silica gel plates (Analtech, Inc.). Most of the reactions were performed under nitrogen atmosphere. ^1H NMR spectra were recorded on Brucker AR, 300-MHz spectrometer: chemical shifts are expressed in δ values (ppm) and coupling constants (J) in Hertz. Mass spectral data were determined on a Brucker-HP Esquire-LC spectrometer (ESI-MS). Fisher scientific Da visil grade 1740 (170–400 mesh) silica gel was used for flash column chromatography. Elemental analysis (C, H, N) was performed by Atlantic Microlab, Inc. (Norcross, GA), and results were within ±0.4% of the theoretical values for the formula given. Yields refer to purified products.

4.1. General procedure for the synthesis of substituted benzamides (3a–l)

Benzoyl chlorides (2.75 mmol) were added portionwise to the stirred solution of 2-halo anilines (2.5 mmol)

and 452 μL of triethyl amine (3.25 mmol) in dry methylene chloride (5 mL) at 0 °C over a period of 15 min, then the mixture was stirred at room temperature for 5–11 h. The reaction mixture was extracted with ethyl acetate, dried, and solvents were removed under reduced pressure. The crude residue was subjected to flash chromatography.

4.1.1. *N*-(2-Bromo-4,6-dimethylphenyl)-3-cyanobenzamide (3a). Yield 61%; ^{1}H NMR ($CDCl_3$): δ 8.24 (s, 1H), 8.19 (d, J = 7.8 Hz, 1H), 7.87(d, J = 7.5 Hz, 1H), 7.65(t, J = 7.7 Hz, 1H,) 7.59(br s, 1H, NH), 7.32 (s, 1H), 7.07 (s, 1H), 2.41 (s, 3H, CH_3), 2.33 (s, 3H, CH_3); MS (ESI): m/z 351.1 $[M+Na]^+$; Anal. Calcd ($C_{16}H_{13}BrN_2O$) C, H, N.

4.1.2. *N*-(2-Chloro-6-methylphenyl)-3-cyanobenzamide (3b). Yield 72%; ^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H, ArH), 8.22 (d, J = 7.8 Hz, 1H, ArH), 7.91 (d, J = 7.8 Hz, 1H, ArH), 7.72 (br s, 1H, ArH), 7.70 (t, J = 7.8 Hz, 1H, ArH) 7.39–7.24 (m, 3H, ArH), 2.38 (s, 3H, CH_3); MS (ESI): m/z 293.2 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{11}ClN_2O$) C,H, N.

4.1.3. *N*-(2-Bromo-4,6-dimethylphenyl)-4-cyanobenzamide (3c). Yield 52%; ^{1}H NMR ($CDCl_3$): δ 8.06 (d, J = 8.4 Hz, 2H, ArH), 7.81 (d, J = 8.4 Hz, 2H, ArH), 7.59 (br s, 1H, NH), 7.33 (s, 1H, ArH), 7.28 (s, 1H, ArH), 2.31 (s, 3H, CH_3), 2.34 (s, 3H, CH_3); MS (ESI): m/z 351 $[M+Na]^+$; Anal. Calcd ($C_{16}H_{13}BrN_2O$) C, H, N.

4.1.4. *N*-(2-Bromo-4,6-dimethylphenyl)4-trifluoromethylbenzamide (3d). Yield 45%; ^{1}H NMR ($CDCl_3$): δ 8.06 (d, J = 8.1 Hz, 2H, ArH), 8.77 (d, J = 8.1 Hz, 2H, ArH), 7.58 (br s, 1H, NH), 7.32 (s, 1H, ArH), 7.07 (s, 1H, ArH), 2.33 (s, 3H, CH_3), 2.31 (s, 3H, CH_3); MS (ESI): m/z 394.3 $[M+Na]^+$; Anal. Calcd ($C_{16}H_{13}BrF_3NO$) C, H, N.

4.1.5. *N*-(2-Chloro-6-methylphenyl)-4-cyanobenzamide (3e). Yield 52%; ^{1}H NMR ($CDCl_3$): δ 8.03 (d, J = 8.4 Hz, 2H, ArH), 7.77 (d, J = 8.4 Hz, 2H, ArH), 7.72 (br s, 1H, NH), 7.33–7.19 (m, 3H, ArH), 2.31 (s, 3H, CH_3); MS (ESI): m/z 293.2 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{11}ClN_2O$) C, H, N.

4.1.6. *N*-(2-Chloro-6-methylphenyl)4-trifluoromethylbenzamide (3f). Yield 75%; ^{1}H NMR ($CDCl_3$): δ 8.04 (d, J = 8.1 Hz, 2H, ArH), 7.75 (d, J = 8.4 Hz, 2H, ArH), 7.32–7.17 (m, 3H, ArH), 2.32 (s, 3H, CH_3); MS (ESI): m/z 336.3 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{11}ClF_3NO$) C, H, N.

4.1.7. *N*-(2-Bromo-4,6-dimethylphenyl)-3-nitrobenzamide (3g). Yield 65%; ^{1}H NMR ($CDCl_3$): δ 8.79 (s, 1H, ArH), 8.45 (d, J = 8.1 Hz, 1H, ArH), 8.31 (d, J = 7.8 Hz, 1H, ArH), 7.72 (t, J = 7.8 Hz, 2H, ArH), 7.32 (s, 1H, ArH), 7.07 (s, 1H, ArH), 2.34 (s, 3H, CH_3), 2.31 (s, 3H, CH_3); MS (ESI): m/z 371.3 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{13}BrN_2O_3$) C, H, N.

4.1.8. *N*-(2-Bromo-4,6-dimethylphenyl)-4-nitrobenzamide (3h). Yield 68%; ^{1}H NMR ($CDCl_3$): δ 8.35 (d, J = 8.7 Hz, 2H, ArH), 8.09 (d, J = 8.7 Hz, 2H, ArH), 7.67 (br s, 1H, NH), 7.31 (s, 1H, ArH), 7.06 (s, 1H, ArH), 2.33 (s, 3H, CH_3), 2.30 (s, 3H, CH_3); MS (ESI): m/z 371.3 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{13}BrN_2O_3$) C, H, N.

4.1.9. *N*-(2-Bromo-4,6-dimethylphenyl)benzamide (3i). Yield 66%; ^{1}H NMR ($CDCl_3$): δ 7.97 (d, J = 9 Hz, 2H, ArH), 7.63–7.49 (m, 3H, ArH), 7.32 (s, 1H, ArH), 7.07(br s, 1H, NH), 2.33 (s, 6H, CH_3); MS (ESI): m/z 327 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{14}BrNO$) C, H, N.

4.1.10. *N*-(2-Chloro-6-methylphenyl)benzamide (3j). Yield 52%; ^{1}H NMR ($CDCl_3$): δ 7.95 (d, J = 7.2 Hz, 2H, ArH), 7.68–7.49 (m, 4H, ArH), 7.33–7.16 (m, 3H, ArH), 2.36 (s, 3H, CH_3); MS (ESI): m/z 268 $[M+Na]^+$; Anal. Calcd ($C_{14}H_{12}ClNO$) C, H, N.

4.1.11. *N*-(2-Bromo-4-trifluoromethylphenyl)benzamide (3k). Yield 70%; ^{1}H NMR ($CDCl_3$): δ 8.77 (d, J = 8.7 Hz, 1H, ArH), 8.63 (br s, 1H, NH), 7.97 (d, J = 7.2 Hz, 2H, ArH), 7.87 (s, 1H, ArH), 7.68–7.54 (m, 5H, ArH); MS (ESI): m/z 365.98 $[M+Na]^+$; Anal. Calcd ($C_{14}H_9BrF_3NO$) C, H, N.

4.1.12. *N*-(5-Acetyl-2-bromo-phenyl)-benzamide (3l). Yield 61%; ^{1}H NMR ($CDCl_3$): δ 9.19 (s, 1H, ArH), 8.54 (br s, 1H, NH) 7.97 (d, J = 6.9 Hz, 2H, ArH), 7.72–7.52 (m, 5H, ArH), 2.67 (s, 3H, CH_3); MS (ESI): m/z 340 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{12}BrNO_2$) C, H, N.

4.1.13. *N*-(2-Bromo-4,6-dimethyl-phenyl)-3-cyano-*N*-(3-cyano-benzoyl)- benzamide (4a). Yield 3.4%; ^{1}H NMR ($CDCl_3$): δ 8.06 (s, 2H, ArH), 8.01 (d, J = 7.8 Hz, 2H, ArH), 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.52 (t, J = 7.8 Hz, 2H, ArH), 7.36 (s, 1H, ArH), 6.95 (s, 1H, ArH), 2.29 (s, 3H, CH_3), 2.19 (s, 3H, CH_3); MS (ESI): m/z 458 $[M+H]^+$; Anal. Calcd ($C_{24}H_{16}BrN_3O_2$) C, H, N.

4.1.14. *N*-(2-Chloro-6-methylphenyl)-3-cyano-*N*-(3-cyanobenzoyl)benzamide (4b). Yield 4.0%; ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 2H, ArH), 8.98 (d, J = 8.1 Hz, 2H, ArH), 7.76 (d, J = 7.8 Hz, 2H, ArH), 7.52 (t, J = 15.9 Hz, 2H, ArH), 7.36 (d, J = 8.1 Hz, 1H, ArH), 7.24 (t, 15.3 Hz, 1H, ArH), 2.24 (s, 3H, CH_3); MS (ESI): m/z 422.3 $[M+Na]^+$; Anal. Calcd ($C_{23}H_{14}ClN_3O_2$) C, H, N.

4.1.15. *N*-(2-Bromo-4,6-dimethylphenyl)-4-cyano-*N*-(4-cyanobenzoyl)benzamide (4c). Yield 3.0%; ^{1}H NMR ($CDCl_3$): δ 7.88 (d, J = 8.4 Hz, 4H, ArH), 7.67 (d, J = 8.4 Hz, 4H, ArH), 7.34 (s, 1H, ArH), 6.94 (s, 1H, ArH), 2.18 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); MS (ESI): m/z 480 $[M+Na]^+$; Anal. Calcd ($C_{24}H_{16}BrN_3O_2$) C, H, N.

4.1.16. *N*-(2-Chloro-6-methylphenyl)-4-cyanobenzoyl) benzamide (4d). Yield 3.0%; ^{1}H NMR ($CDCl_3$): δ 7.85 (d, J = 8.4 Hz, 4H, ArH), 7.66 (d, J = 8.4 Hz, 4H, ArH), 7.35 (d, J = 7.8 Hz, 1H, ArH), 7.23 (t, J = 7.8 Hz, 1H, ArH), 7.12 (d, J = 7.2 Hz, 1H, ArH), 2.28 (s, 3H, CH_3); MS (ESI): m/z 422.1 $[M+Na]^+$; Anal. Calcd ($C_{23}H_{14}ClN_3O_2$) C, H, N.

4.1.17. *N*-(2-Chloro-6-methylphenyl)-4-trifluoromethyl-*N*-(4-trifluoromethylbenzoyl)benzamide (4e). Yield 2.5%; ^{1}H NMR ($CDCl_3$): δ 7.90 (d, J = 8.1 Hz, 4H, ArH), 7.62 (d, J = 8.4 Hz, 4H, ArH), 7.35 (d, J = 7.8 Hz, 1H, ArH), 7.21 (t, J = 7.8 Hz, 1H, ArH), 7.12 (d, J = 7.6 Hz, 1H, ArH), 2.26 (s, 3H, CH_3); MS (ESI): m/z 508.1 $[M+Na]^+$; Anal. Calcd ($C_{23}H_{14}ClF_6NO_2$) C, H, N.

4.1.18. *N*-(2-Bromo-4,6-dimethyl-phenyl)-3-nitro-*N*-(3-nitro-benzoyl)- benzamide (4f). Yield 2.0%; ^{1}H NMR ($CDCl_3$)δ 8.65 (s, 2H, ArH), 8.35 (d, J = 8.4 Hz, 2H, ArH), 8.14 (d, J = 7.5 Hz, 2H, ArH), 7.61 (t, J = 8.1 Hz, 2H, ArH), 7.36 (s, 1H, ArH), 6.96 (s, 1H, ArH), 2.28 (s, 3H, CH_3), 2.31 (s, 3H, CH_3); MS (ESI): m/z 520 $[M+Na]^+$; Anal. Calcd ($C_{22}H_{16}BrN_3O_6$) C, H, N.

4.1.19. *N*-(2-Bromo-4,6-dimethylphenyl)-4-nitro-*N*-(4- nitrobenzoyl)benzamide (4g). Yield 2.5%; ^{1}H NMR ($CDCl_3$)δ 8.24 (d, J = 8.7 Hz, 4H, ArH), 7.96 (d, J = 8.7 Hz, 4H, ArH), 7.35 (s, 1H, ArH), 6.94 (s, 1H, ArH), 2.28 (s, 3H, CH_3), 2.20 (s, 3H, CH_3); MS (ESI): m/z 520 $[M+Na]^+$; Anal. Calcd ($C_{22}H_{16}BrN_3O_6$) C, H, N.

4.2. General procedure for synthesis of 5*H*-phenanthridin-6-ones (5a–j)

Substituted benzamides (1.31 mmol), palladium(II) acetate (29.34 mg, 0.131 mmol), and anhydrous sodium carbonate (161 mg, 1.52 mmol) in dry *N*,*N*-dimethyl acetamide (6 mL) were heated together at 165–170 °C under nitrogen for 11–23 h. The resultant mixture was cooled to room temperature, poured to cold water, and extracted with ethyl acetate (3× 5 mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4, solvents were evaporated under reduced pressure, and crude residue was purified by flash chromatography.

4.2.1. 2,4-Dimethyl-6-oxo-5,6-phenanthridine-8-carbonitrile (5a). Yield 30%; ^{1}H NMR ($CDCl_3$): δ 9.62 (br s, 1H, NH), 8.63 (s, 1H, ArH), 8.24 (d, J = 8.4 Hz, 1H, ArH), 7.81 (d, J = 8.4 Hz, 1H, ArH), 7.28 (s, 1H, ArH), 7.09 (s, 1H, ArH), 2.29 (s, 3H, CH_3), 2.34 (s, 6H, CH_3); MS (ESI): m/z 247.3 $[M-H]^-$; Anal. Calcd ($C_{16}H_{12}N_2O$) C, H, N.

4.2.2. 4-Methyl-6-oxo-5,6-dihydrophenanthridine-8-carbonitrile (5b). Yield 44%; ^{1}H NMR ($CDCl_3$): δ 11.01 (br s, 1H, NH), 8.70 (t, J = 8.4 Hz, 1H, ArH), 8.66 (s, 1H, ArH), 8.34 (d, J = 8.1 Hz, 1H, ArH), 8.23 (d, J = 8.7 Hz, 1H, ArH), 7.45 (d, J = 7.5 Hz, 1H, ArH), 7.23 (t, J = 7.8 Hz, 1H, ArH), 2.48 (s, 3H, CH_3); MS (ESI): m/z 235.2 $[M+H]^+$; Anal. Calcd ($C_{15}H_{10}N_2O$) C, H, N.

4.2.3. 2,4-Dimethyl-6-oxo-5,6-hydrophenanthridine-9-carbonitrile (5c). Yield 24%; ^{1}H NMR ($CDCl_3$): δ 10.95 (br s, 1H, NH), 9.1 (s, 1H, ArH), 8.44 (d, J = 8.1 Hz, 1H, ArH), 8.23 (s, 1H, ArH), 8.00 (d, J = 8.2 Hz, 1H, ArH), 7.26 (s, 1H, ArH), 2.39 (s, 3H, CH_3), 2.45 (s, 6H, CH_3); MS (ESI): m/z271 $[M+Na]^+$; Anal. Calcd ($C_{16}H_{12}N_2O$) C, H, N.

4.2.4. 2,4-Dimethyl-9-trifluoromethyl-5*H*-phenanthridin-6-one (5d). Yield 28%; ^{1}H NMR ($CDCl_3$): δ 8.64 (d, J = 8.1 Hz, 1H, ArH), 8.54 (s, 1H, ArH), 7.91 (s, 1H, ArH), 7.81 (d, J = 8.4 Hz, 1H, ArH), 7.23 (s, 1H, ArH), 2.48 (s, 3H, CH_3), 2.47 (s, 6H, CH_3); MS (ESI): m/z 314.1, $[M+Na]^+$; Anal. Calcd ($C_{16}H_{12}F_3NO$) C, H, N.

4.2.5. 4-Methyl-6-oxo-5,6-dihydro-phenanthridine-9-carbonitrile (5e). Yield 23%; ^{1}H NMR (DMSO-d_6, CD_3OD): δ 9.09 (s, 1H, ArH), 8.46 (d, J = 7.8 Hz, 1H, ArH), 8.37 (d, J = 7.1 Hz, 1H, ArH), 8.00 (d, J = 8.4 Hz, 1H, ArH), 7.42 (d, J = 7.5 Hz, 1H, ArH), 7.19 (t, J = 7.2 Hz, 1H, ArH), 2.16 (s, 3H, CH_3); MS (ESI): m/z 257.2 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{10}N_2O$) C, H, N.

4.2.6. 4-Methyl-9-trifluoromethyl-5*H*-phenanthridin-6-one (5f). Yield 38%; ^{1}H NMR (DMSO-d_6, CD_3OD): δ 8.77 (s, 1H, ArH), 8.59 (d, J = 9.0 Hz, 1H, ArH), 8.32 (d, J = 8.0 Hz, 1H, ArH), 7.91 (d, J = 9.0 Hz, 1H, ArH), 7.42 (d, J = 7.0 Hz, 1H, ArH), 7.27 (t, J = 8.0 Hz, 1H, ArH), 2.50 (s, 3H, CH_3); MS (ESI): m/z 300 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{10}F_3NO$) C, H, N.

4.2.7. 2,4-Dimethyl-5*H*-phenanthridin-6-one (5g). Yield 30%; ^{1}H NMR ($CDCl_3$): δ 8.81 (br s, 1H, NH), 8.53 (d, J = 8.1 Hz, 1H, ArH), 8.30 (d, J = 8.1 Hz, 1H, ArH), 7.90 (s, 1H, ArH), 7.79 (t, J = 8.1 Hz, 1H, ArH), 7.60 (t, J = 8.1 Hz, 1H, ArH), 7.17 (s, 1H, ArH), 2.47 (s, 6H, CH_3); MS (ESI): m/z 224.5 $[M+H]^+$; Anal. Calcd ($C_{15}H_{13}NO$) C, H, N.

4.2.8. 4-Methyl-5*H*-phenanthridin-6-one (5h). Yield 30%; ^{1}H NMR ($CDCl_3$)δ 8.53 (br s, 1H, NH), 8.51 (s, 1H, ArH), 8.30 (d, J = 8.1 Hz, 1H, ArH), 8.12 (d, J = 8.1 Hz, 1H, ArH), 7.80 (t, J = 7.2 Hz, 1H, ArH), 7.60 (t, J = 7.5 Hz, 1H, ArH), 7.35 (d, J = 7.9 Hz, 1H, ArH), 7.21 (t, J = 7.8 Hz, 1H, ArH), 2.48 (s, 6H, CH_3); MS (ESI): m/z 232 $[M+Na]^+$; Anal. Calcd ($C_{14}H_{11}NO$) C, H, N.

4.2.9. 2-Trifluoromethyl-5*H*-phenanthridin-6-one (5i). Yield 53%; ^{1}H NMR ($CDCl_3$): δ 11.43 (br s, 1H, NH), 8.39 (d, J = 8.1 Hz, 1H, ArH), 8.32 (s, 1H, ArH), 8.21 (d, J = 8.1 Hz, 1H, ArH), 7.73 (t, J = 7.2 Hz, 1H, ArH), 7.54 (t, J = 7.5 Hz, 2H, ArH), 7.37 (d, J = 8.7 Hz, 1H, ArH); MS (ESI): m/z 286 $[M+Na]^+$; Anal. Calcd ($C_{14}H_8F_3NO$) C, H, N.

4.2.10. 3-Acetyl-5H-phenanthridin-6-one (5j). Yield 30%; ^{1}H NMR (CD_3OD) δ 8.53–8.40 (m, 3H, ArH), 7.96–7.86 (m, 3H, ArH), 7.70 (t, J = 7.2 Hz, 1H, ArH), 2.67 (s, 3H, CH_3); MS (ESI): m/z 236 $[M-H]^-$; Anal. Calcd ($C_{15}H_{11}NO_2$) C, H, N.

4.2.11. Synthesis of 2-methoxy-5*H*-phenanthridin-6-one (8). 2-Bromo-5*H*-phenanthridin-6-one (60 mg, 0.216 mmol), cuprous iodide (41 mg, 216 mmol), and sodium methoxide (prepared freshly by dissolving 80 mg of sodium metal in 5 mL methanol) were refluxed gently in anhydrous DMF nearly for 5 h. Reaction mixture was cooled to room temperature and extracted with ethyl

acetate, dried, and solvents were removed under reduced pressure. Crude residue was further purified by flash chromatography to get 2-methoxy-5*H*-phenanthridin-6-one (41 mg, 83% yield).

^{1}H NMR (C_6D_6O, DMSO-d_6): δ 11.43 (br s, 1H, NH), 8.52 (d, J = 8.4 Hz, 1H, ArH), 8.41 (dd, J = 1.5, 1.2 Hz, 1H, ArH), 7.88–7.82 (m, 2H, ArH) 7.64 (t, J = 8.1 Hz, 1H, ArH), 7.38 (d, J = 9.0 Hz, 1H, ArH), 7.14 (dd, J = 2.7, 2.7 Hz, 1H, ArH), 3.92 (s, 3H, OCH_3); MS (ESI): m/z 248 $[M+Na]^+$; Anal. Calcd ($C_{14}H_{11}NO_2$) C, H, N.

4.2.12. Synthesis of 6-oxo-5,6-dihydro-phenanthridine-2-carbonitrile (9). 2-Bromo-5*H*-phenanthridin-6-one (150 mg, 0.54 mmol) and cuprous cyanide (58 mg, 0.648 mmol) were heated with stirring in *N*,*N*-dimethyl acetamide (4 mL) at 150–155 °C for a day. After cooling the reaction mixture to room temperature, 5 mL cold water was added to the reaction flask and separated solid was filtered. Column chromatography of the crude product gave 6-oxo-5,6-dihydro-phenanthridine-2-carbonitrile as white powder (90 mg, 75% yield).

^{1}H NMR (DMSO-d_6): δ 12.00 (br s, 1H, NH), 8.92 (s, 1H, ArH), 8.59 (d, J = 8.1 Hz, 1H, ArH), 8.28 (d, J = 7.5 Hz, 1H, ArH), 7.85 (t, d, J = 6.6, 8.7 Hz, 2H, ArH), 7.67 (t, J = 7.8 Hz, 1H, ArH), 7.42 (d, J = 8.4 Hz, 1H, ArH); MS (ESI): m/z 219 $[M-H]^-$; Anal. Calcd ($C_{14}H_8N_2O$) C, H, N.

4.2.13. Synthesis of 6-oxo-5,6-dihydro-phenanthridine-2-carboxylic acid (10). To a suspension of 6-oxo-5,6-dihydro-phenanthridine-2-carbonitrile (100 mg, 0.454 mmol) in ethanol (10 mL), sodium hydroxide (100 mg) was added and reaction mixture was refluxed for 21 h. The cooled reaction mixture was added to ice-cold water and carefully acidified with dilute hydrochloric acid, separated white precipitate was filtered and washed with water repeatedly and air-dried to get sufficiently pure 6-oxo-5,6-dihydro-phenanthridine-2-carboxylic acid in 30% yield.

^{1}H NMR (DMSO-d_6): δ 13.00 (br s, 1H, OH), 11.89 (s, 1H, NH), 8.91 (s, 1H, ArH), 8.59 (d, J = 7.8 Hz, 1H, ArH), 8.32 (d, J = 8.1 Hz, 1H, ArH), 8.13 (s, 1H, ArH), 8.01 (dd, J = 0.9, 1.2 Hz, 1H, ArH), 7.91 (t, J = 7.5 Hz, 1H, ArH), 7.68 (t, J = 7.5 Hz, 1H, ArH), 7.38 (d, J = 8.4 Hz, 1H, ArH); MS (ESI): m/z 238 $[M-H]^-$; Anal. Calcd ($C_{14}H_9NO_3$) C, H, N.

4.3. General procedure for synthesis of N-substituted 2-amino-5*H*-phenanthridin-6-ones (12a–d)

Acid chlorides (ethyl iodide in case of **12a**) (0.253 mmol) were added portionwise to the stirred solution of 2-amino-5*H*-phenanthridin-6-one (0.23 mmol) and triethyl amine (42 μL, 0.299 mmol) in dry DMF (5 mL) at 0 °C over a period of 15 min. After the addition was complete, the reaction mixture was stirred at room temperature for 2–5 h (28 h in case of ethyl iodide). Reaction mixture was extracted with ethyl acetate, dried, and solvents were removed under reduced pressure. The crude residue was purified by flash chromatography.

4.3.1. 2-Ethylamino-5*H*-phenanthridin-6-one (12a). Yield 50%; ^{1}H NMR (DMSO-d_6): δ 11.46 (br s, 1H, NH), 8.62 (d, J = 8.1 Hz, 1H, ArH), 8.24 (d, J = 8.1 Hz, 1H, ArH), 7.78 (t, J = 6.9 Hz, 1H, ArH), 7.61 (t, J = 7.2 Hz, 1H, ArH), 7.40–7.26 (m, 2H, ArH), 6.88 (d, J = 8.7 Hz, 1H, ArH), 3.70 (br s, 1H, NH), 3.28 (q, J = 6.9, 7.2, 6.9 Hz, 2H, CH_2), 1.34 (t, J = 7.2, Hz, 3H, CH_3); MS (ESI): m/z 261 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{14}N_2O$) C, H, N.

4.3.2. *N*-(6-Oxo-5,6-dihydro-phenanthridin-2-yl)-acetamide (12b). Yield 50%; ^{1}H NMR (DMSO-d_6): δ 11.64 (br s, 1H, NH), 11.06 (br s, 1H, NH), 8.61 (s, 1H, ArH), 8.32 (d, J = 7.8 Hz, 1H, ArH), 8.22 (d, J = 7.5 Hz, 1H, ArH), 7.88 (t, J = 7.2 Hz, 1H, ArH), 7.64 (d t, J = 7.8, 8.1 Hz, 2H, ArH), 7.29 (d, J = 8.4 Hz, 1H, ArH), 2.07 (s, 3H, CH_3); MS (ESI): m/z 251 $[M-H]^-$; Anal. Calcd ($C_{15}H_{12}N_2O_2$) C, H, N.

4.3.3. *N*-(6-Oxo-5,6-dihydro-phenanthridin-2-yl)-benzamide (12c). Yield 60%; ^{1}H NMR (DMSO-d_6): δ 11.71 (br s, 1H, NH), 10.38 (br s, 1H, NH), 8.79 (s, 1H, ArH), 8.32 (t, J = 8.4 Hz, 2H, ArH), 8.01 (d, J = 6.6 Hz, 2H, ArH), 7.93–7.86 (m, 2H, ArH), 7.69–7.53 (m, 4H, ArH), 7.35 (d, J = 8.7 Hz, 1H, ArH); MS (ESI): m/z 337 $[M+Na]^+$; Anal. Calcd ($C_{20}H_{14}N_2O_2$) C, H, N.

4.3.4. *N*-(6-Oxo-5,6-dihydro-phenanthridin-2-yl)-benzenesulfonamide (12d). Yield 85%; ^{1}H NMR (DMSO-d_6, CD_3OD): δ 8.79 (s, 1H, ArH), 8.51 (d, J = 8.4 Hz, 1H, ArH), 8.14 (d, J = 8.1 Hz, 1H, ArH), 8.05–7.72 (m, 9H, ArH), 7.45 (d, J = 8.7 Hz, 1H, ArH), 7.22 (d, J = 8.7 Hz, 1H, ArH); MS (ESI): m/z 348.9 $[M-H]^-$; Anal. Calcd ($C_{19}H_{14}N_2O_3S$) C, H, N.

4.4. General procedures for synthesis of compounds 13a–b, 14a–b

Method A. 3 or 4-Bromomethylbenzonitriles (37.24 mg, 0.19 mmol) were added to the stirred solution of 2-amino-5*H*-phenanthridin-6-one (40 mg, 0.19 mmol) and triethyl amine (29 μL, 0.209 mmol) in dry dimethyl formamide (4 mL). After the addition was complete, the reaction mixture was stirred at room temperature for 4 h. Mixture was extracted with ethyl acetate, dried over anhydrous Na_2SO_4, and solvents were removed under reduced pressure. Purification of all the crude products was done by column chromatography.

Method B. 3 or 4-Bromomethylbenzonitriles (37.24 mg, 0.19 mmol) were added to the stirred solution of substituted 2-amino-5*H*-phenanthridin-6-one (40 mg, 0.19 mmol) and pyridine (17 μL, 0.209 mmol) in dry dimethyl formamide (5 mL). The reaction mixture was stirred for 4 h at room temperature. Workup and purification of the reaction mixture is the same as above.

Method C. 3 or 4-Bromomethylbenzonitriles (90 mg, 0.46 mmol), 2-amino-5*H*-phenanthridin-6-one (100 mg,

0.46 mmol), and potassium carbonate (126 mg, 0.92 mmol) were refluxed for 4 h in dry ethanol (10 mL). Reaction mixture was filtered hot and solvents removed under reduced pressure. Workup and purification of the reaction mixture is the same as above.

4.4.1. 3-[(6-Oxo-5,6-dihydro-phenanthridin-2-ylamino)-methyl]-benzonitrile (13a). Yield 60%; ^{1}H NMR (DMSO-d_6): δ 11.23 (br s, 1H, NH), 8.29 (d, J = 7.5 Hz, 1H, ArH), 8.09 (d, J = 8.1 Hz, 1H, ArH), 7.75–7.65 (m, 3H, ArH), 7.53-7.42 (m, 3H, ArH), 7.28 (s, 1H, ArH), 7.12 (d, J = 9.0 Hz, 1H, ArH), 6.78 (dd, J = 2.4, 2.4 Hz, 1H, ArH), 5.97 (t, J = 6.0 Hz, 1H, NH), 4.41 (d, J = 1.9 Hz, 2H, CH_2); MS (ESI): m/z 348 $[M+Na]^+$; Anal. Calcd ($C_{21}H_{15}N_3O$) C, H, N.

4.4.2. 4-[(6-Oxo-5,6-dihydro-phenanthridin-2-ylamino)-methyl]-benzonitrile (13b). Yield 60%; ^{1}H NMR (DMSO-d_6): δ 11.30 (br s, 1H, NH), 8.22 (t, J = 8.4 Hz, 2H, ArH), 7.74 (sd, J = 8.1 Hz, 3H, ArH), 7.55 (m, 4H, ArH), 7.34 (s, 1H, ArH), 7.08 (d, J = 8.7 Hz, 1H, ArH), 6.83 (dd, J = 2.1, 2.4 Hz, 1H, ArH), 6.30 (t, J = 6.0 Hz, 1H, NH), 4.5 (d, J = 1.9 Hz, 2H, CH_2); MS (ESI): m/z 348 $[M+Na]^+$; Anal. Calcd ($C_{21}H_{15}N_3O$) C, H, N.

4.4.3. *N*-[3-(Cyano phenyl methyl)]3-[(6-oxo-5,6-dihydro-phenanthridin-2-yl amino)methyl]benzonitrile (14a). Yield 5%; ^{1}H NMR (DMSO-d_6): δ 9.82 (br s, 1H, NH), 8.54 (d, J = 8.1 Hz, 1H, ArH), 7.96 (d, J = 7.8 Hz, 1H, ArH), 7.74 (t, J = 7.2 Hz, 1H, ArH), 7.407.65–7.45 (m, 10H, ArH), 7.17 (d, J = 8.7 Hz, 1H, ArH), 6.96 (d, J = 7.5 Hz, 1H, ArH), 4.74 (s, 4H, CH_2); MS (ESI): m/z 463 $[M+Na]^+$; Anal. Calcd ($C_{29}H_{20}N_4O$) C, H, N.

4.4.4. *N*-[4-(Cyano phenyl methyl)]4-[(6-oxo-5,6-dihydro-phenanthridin-2-yl amino)methyl]benzonitrile (14b). Yield 6%; ^{1}H NMR (DMSO-d_6): δ 11.31 (br s, 1H, NH), 8.27 (d, J = 7.8 Hz, 1H, ArH), 8.02 (sd, J = 8.1 Hz, 3H, ArH), 7.64 (d, J = 8.1 Hz, 4H, ArH), 7.55–7.39 (m, 6H, ArH), 7.15 (d, J = 8.7 Hz, 1H, ArH), 6.85 (dd, J = 2.1, 2.4 Hz, 1H, ArH), 4.78 (s, 4H, CH_2); MS (ESI): m/z 463 $[M+Na]^+$; Anal. Calcd ($C_{29}H_{20}N_4O$) C, H, N.

4.4.5. 2,4-Dioxo-4-(6-oxo-5,6-dihydro-phenanthridin-3-yl)-butyric acid ethyl ester (15). To a solution of 3-acetyl-5*H*-phenanthridin-6-one (80 mg, 0.337 mmol) in dry dioxane, potassium *tert*-butoxide (378 mg, 3.37 mmol) and diethyl oxalate (59.5 μL, 0.438 mmol) were added with stirring. The reaction mixture was gently refluxed overnight, cooled to room temperature and carefully acidified with dilute HCl. Extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and solvents were removed under reduced pressure. The crude product was used with out further purification in next step.

4.5. General procedure for compounds 17, 20, and 26a–f

A freshly prepared sodium methoxide (231 mg, 4.278 mmol) in methanol (8 mL) was added to the mixture of acetyl derivatives (1.337 mmol) and diethyl oxalate (236 μL, 1.738 mmol), and the reaction mixture was stirred for 3–5 h at room temperature. Reaction mixture was poured to cold water and carefully acidified with dilute HCl. The mixture was extracted with ethyl acetate, dried over anhydrous Na_2SO_4, and solvents were removed under reduced pressure. The crude product was used without further purification in next step.

4.5.1. 4-(3-Benzoylamino-4-bromo-phenyl)-2,4-dioxo-butyric acid methyl ester (17). Yield 36%; ^{1}H NMR (DMSO-d_6): δ 9.92(br s, 1H, NH) 7.90–8.20 (m, 4H, ArH), 7.80-7.40 (m, 6H, ArH, C=CH), 6.55 (br s, 1H, OH/or C=CH), 3.73 (s, 3H, OCH3); MS (ESI): m/z 402 $[M-H]^-$; Anal. Calcd ($C_{18}H_{14}BrNO_5$) C, H, N.

4.5.2. 4′-(3-Methoxycarbonyl-3-oxo-propionyl)-2′-nitro-biphenyl-2-carboxylic acid methyl ester (20). Yield 47%; ^{1}H NMR ($CDCl_3$): δ 8.70 (s, 1H, ArH), 8.24 (dd, J = 1.8, 1.5 Hz, 1H, ArH), 8.13 (d, J = 7.5 Hz, 1H, ArH), 7.63 (t, J = 7.5 Hz, 1H, ArH), 7.53 (t, J = 7.8 Hz, 1H, ArH), 7.44 (d, J = 8.1 Hz, 1H, ArH), 7.26 (s, 1H, C=CH), 7.23 (d, J = 7.5 Hz, 1H, ArH), 7.15 (br s, 1H, OH/or C=CH), 3.98 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3).

^{13}C NMR ($CDCl_3$): δ ppm 187.13, 169.98, 166.00, 161.65, 148.00, 141.84, 138.49, 134.45, 131.96, 131.70, 130.67, 130.22, 128.95, 128.12, 127.63, 122.82, 97.46, 52.85, 51.64; MS (ESI): m/z 408 $[M+Na]^+$; Anal. Calcd ($C_{19}H_{15}NO_8$) C, H, N.

4.6. General procedure for compounds 16, 18, 21, and 27a–k

Diketo ester derivative (0.1 mmol) was stirred with 80 mg of sodium hydroxide in 20 mL of methanol for 3–5 h at room temperature. Poured to ice-cold water and acidified with dilute HCl. Precipitate was extracted with ethyl acetate, dried, and evaporated to get the diketo acids with good purity.

4.6.1. 2,4-Dioxo-4-(6-oxo-5,6-dihydro-phenanthridin-3-yl)-butyric acid (16). Yield 72%; ^{1}H NMR (DMSO-d_6): δ 11.82 (br s, 1H, NH), 8.61 (d, J = 9.0 Hz, 1H, ArH), 8.56 (d, J = 9.0 Hz, 1H, ArH), 8.36 (d, J = 9.0 Hz, 1H, ArH), 8.04 (s, 1H, ArH), 7.96–7.86 (m, 3H, ArH, C=CH), 7.78–7.68 (m, 2H, ArH), 7.00 (br s, 1H, OH); MS (ESI): m/z 307.8 $[M-H]^-$; Anal. Calcd ($C_{17}H_{11}NO_5$) C, H, N.

4.6.2. 4-(3-Benzoylamino-4-bromo-phenyl)-2,4-dioxo-butyric acid (18). Yield 50%; ^{1}H NMR ($CDCl_3$): δ 9.22 (br s, 1H, NH), 8.54 (br s, 1H, ArH) 7.96 (d, J = 7.2 Hz, 2H, ArH), 7.77–7.50 (m, 5H, ArH, C=CH), 7.26 (s, 1H, ArH), 7.20 (s, 1H, OH/or C=CH); MS (ESI): m/z 412 $[M+Na]^+$; Anal. Calcd ($C_{17}H_{12}BrNO_5$) C, H, N.

4.6.3. 4′-(3-Carboxy-3-oxo-propionyl)-2′-nitro-biphenyl-2-carboxylic acid methyl ester (21). Yield 40%; ^{1}H NMR ($CDCl_3$, DMSO-d_6): δ 8.71 (s, 1H, ArH), 8.16 (d, J = 7.8 Hz, 1H, ArH), 8.03 (d, J = 7.8 Hz, 1H, ArH), 7.60–7.34 (m, 5H, ArH, C=CH), 7.16 (d, J = 9.0 Hz, 1H, ArH), 7.08 (br s, 1H, OH/or C=CH),

3.60 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$): δ ppm 187.07, 171.04, 165.62, 162.62, 147.72, 141.34, 138.32, 134.55, 131.85, 131.61, 130.65, 129.97, 128.89, 127.93, 127.42, 122.49, 97.24, 51.45; MS (ESI): *m/z* 370 $[M-H]^-$; Anal. Calcd ($C_{18}H_{13}NO_8$) C, H, N.

4.6.4. 4′-Acetyl-2′-amino-biphenyl-2-carboxylic acid methyl ester (22). 4′-Acetyl-2′-nitro-biphenyl-2-carboxylic acid methyl ester **26** (50 mg, 0.167 mmol), hydrazine hydrate (20 μL, 0.334 mmol), and Raney nickel (8 mg) were stirred in methanol for 20 h. Mixture was filtered through Celite and solvents were removed under reduced pressure. The crude residue was purified by flash chromatography.

Yield 60%; ^1H NMR ($CDCl_3$): δ 8.40 (d, $J = 1.8$ Hz, 1H, ArH), 8.09 (dd, $J = 1.2$, 1.2 Hz, 1H, ArH), 7.95 (dd, $J = 1.8$, 1.8 Hz, 1H, ArH), 7.60 (t, $J = 7.5$ Hz, 1H, ArH), 7.49 (t, $J = 7.8$ Hz, 1H, ArH), 7.25 (d, $J = 8.1$ Hz, 2H, ArH), 5.57 (br s, 2H, NH_2), 3.68 (s, 3H, OCH_3), 2.19 (s, 3H, $COCH_3$); MS (ESI): *m/z* 392 $[M+Na]^+$; Anal. Calcd ($C_{16}H_{15}NO_3$) C, H, N.

4.6.5. 4-(4-Morpholin-4-yl-phenyl)-2,4-dioxo-butyric acid (27a). Yield 60%; ^1H NMR (C_3D_6O): δ 8.02 (d, $J = 9.3$ Hz, 2H, ArH), 7.07 (s, d, $J = 9.0$ Hz, 3H, CH, ArH), 3.81 (t, $J = 4.8$, 5.1 Hz, 4H, CH_2), 3.43 (t, $J = 5.1$, 4.8 Hz, 4H, CH_2); MS (ESI): *m/z* 300 $[M+Na]^+$; Anal. Calcd ($C_{14}H_{15}NO_5$) C, H, N.

4.6.6. 4-Naphthalen-2-yl-2,4-dioxo-butyric acid (27b). Yield 40%; ^1H NMR (C_3D_6O): δ 8.84 (s, 1H, ArH), 8.20–8.01 (m, 4H, ArH), 7.73–7.64 (m, 2H, ArH), 7.36 (s, 1H, CH); MS (ESI): *m/z* 265 $[M+Na]^+$; Anal. Calcd ($C_{14}H_{10}O_4$) C, H, N.

4.6.7. 4-(6-Methyl-naphthalen-2-yl)-2,4-dioxo-butyric acid (27c). Yield 56%; ^1H NMR (C_3D_6O): δ 8.76 (s, 1H, ArH), 8.11-8.07 (m, 2H, ArH), 7.96 (d, $J = 8.7$ Hz, 1H, ArH), 7.79 (s, 1H, CH), 7.50 (dd, $J = 1.5$, 1.5 Hz, 1H, CH), 7.33 (s, 1H, CH), 2.55 (s, 3H, CH_3); MS (ESI): *m/z* 279 $[M+Na]^+$; Anal. Calcd ($C_{15}H_{12}O_4$) C, H, N.

4.6.8. 4-Anthracen-2-yl-2,4-dioxo-butyric acid (27d). Yield 92%; ^1H NMR (C_3D_6O): δ 9.06 (s, 1H, ArH), 8.89 (s, 1H, ArH), 8.66 (s, 1H, ArH), 8.26–8.06 (m, 5H, ArH), 7.64–7.58 (m, 2H, ArH), 7.42 (s, 1H, CH); MS (ESI): *m/z* 290.8 $[M-H]^-$; Anal. Calcd ($C_{18}H_{12}O_4$) C, H, N.

4.6.9. 4-Anthracen-9-yl-2,4-dioxo-butyric acid (27e). Yield 77%; ^1H NMR (C_3D_6O): δ 8.79 (s, 1H, ArH), 8.21–8.13 (m, 4H, ArH), 7.64–7.59 (m, 5H, ArH), 6.82 (s, 1H, CH); MS (ESI): *m/z* 315.1 $[M+Na]^+$; Anal. Calcd ($C_{18}H_{12}O_4$) C, H, N.

4.6.10. 2,4-Dioxo-4-pyren-1-yl-butyric acid (27f). Yield 60%; ^1H NMR (C_3D_6O): δ 8.97 (d, $J = 9.3$ Hz, 1H, ArH), 8.53–8.15 (m, 9H, ArH), 7.23 (s, 1H, CH); MS (ESI): *m/z* 339 $[M+Na]^+$; Anal. Calcd ($C_{20}H_{12}O_4$) C, H, N.

4.6.11. 2,4-Dioxo-4-phenanthren-9-yl-butyric acid (27g). Yield 70%; ^1H NMR (C_3D_6O): δ 8.94 (d, $J = 8.1$ Hz, 1H, ArH), 8.89 (d, $J = 8.1$ Hz, 1H, ArH), 8.67 (d, $J = 7.8$ Hz, 1H, ArH), 8.49 (s, 1H, ArH), 8.23 (d, $J = 7.8$ Hz, 1H, ArH), 7.87–7.72 (m, 4H, ArH), 7.16 (s, 1H, CH); MS (ESI): *m/z* 315 $[M+Na]^+$; Anal. Calcd ($C_{18}H_{12}O_4$) C, H, N.

4.6.12. 2,4-Dioxo-4-phenanthren-3-yl-butyric acid (27h). Yield 62%; ^1H NMR (C_3D_6O): δ 9.02 (d, $J = 8.7$ Hz, 1H, ArH), 8.95–8.92 (m, 1H, ArH), 8.86 (d, $J = 2.1$ Hz, 1H, ArH), 8.37 (dd, $J = 1.8$, 2.1 Hz, 1H, ArH), 8.10–7.97 (m, 3H, ArH), 7.80–7.76 (m, 2H, ArH), 7.40 (s, 1H, CH); MS (ESI): *m/z* 315.1 $[M+Na]^+$; Anal. Calcd ($C_{18}H_{12}O_4$) C, H, N.

4.6.13. 2,4-Dioxo-4-phenanthren-2-yl-butyric acid (27i). Yield 55%; ^1H NMR (C_3D_6O, DMSO-d_6): δ 9.36 (s, 1H, ArH), 8.99 (d, $J = 7.2$ Hz, 1H, ArH), 8.14–8.02 (m, 5H, ArH), 7.76–7.66 (m, 2H, ArH); MS (ESI): *m/z* 315.1 $[M+Na]^+$; Anal. Calcd ($C_{18}H_{12}O_4$) C, H, N.

4.6.14. 4-(2-Methyl-10-oxo-10,10a-dihydro-8aH-9-oxa-1-aza-anthracen-3-yl)-2,4- dioxo-butyric acid (27j). Yield 50%; ^1H NMR (DMSO-d_6): δ 8.75 (s, 1H, ArH), 8.23 (d, $J = 8.1$ Hz, 1H, ArH), 7.96 (t, $J = 8.7$ Hz, 1H, ArH), 7.75 (d, $J = 8.7$ Hz, 1H, ArH), 7.56 (t, $J = 7.8$ Hz, 1H, ArH), 6.88 (s, 1H, CH), 2.83 (s, 3H, CH_3); MS (ESI): *m/z* 324 $[M-H]^-$; Anal. Calcd ($C_{17}H_{13}NO_6$) C, H, N.

4.6.15. 4-(2,6-Dimethyl-10-oxo-10,10a-dihydro-8a*H*-9-oxa-1-aza- anthracen-3-yl)-2,4-dioxo-butyric acid (27k). Yield 45%; ^1H NMR (C_3D_6O, DMSO-d_6): δ 9.31 (s, 1H, ArH), 8.48 (s, 1H, ArH), 8.23 (d, $J = 8.4$ Hz, 1H, ArH), 8.08 (d, $J = 8.4$ Hz, 1H, ArH), 7.42 (s, 1H, CH), 3.30 (s, 3H, CH_3), 2.95 (s, 3H, CH_3); MS (ESI): *m/z* 338 $[M-H]^-$; Anal. Calcd ($C_{18}H_{15}NO_6$) C, H, N.

4.7. Biological assays

4.7.1. Materials, chemicals, and enzymes. All compounds were dissolved in DMSO and the stock solutions were stored at −20 °C. The $\gamma[^{32}P]$-ATP was purchased from either Amersham Biosciences or ICN. The expression systems for wild-type IN were a generous gift from Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD. Cell lines were obtained from American Tissue Type Culture (Rockville, MD).

4.7.2. Preparation of oligonucleotide substrates. The oligonucleotides 21top, 5′-GTGTGGAAAATCTCTAGCAGT-3′, and 21bot, 5′-ACTGCTAGAGATTTTCCACAC-3′, were purchased from the Norris Cancer Center Microsequencing Core Facility (University of Southern California) and purified by UV shadowing on polyacrylamide gel. To analyze the extent of 3′-end processing and strand transfer using 5′-end labeled substrates, 21top was 5′-end labeled using T_4 polynucleotide kinase (Epicentre, Madison, WI) and $\gamma[^{32}P]$-ATP

(Amersham Biosciences or ICN). The kinase was heat-inactivated and 21-bot was added in 1.5 M excess. The mixture was heated at 95 °C, allowed to cool slowly at room temperature, and run through a G25 mini spin column (USA Scientific) to separate double-double stranded oligonucleotide from unincorporated material.

4.7.3. Integrase assays. To determine the extent of 3′-processing and strand transfer, wild-type IN was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin, 10 mM 2-mercaptoethanol, 10% dimethylsulfoxide, and 25 mM MOPS, pH 7.2) at 30 °C for 30 min. An aliquot (5 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M Tris–borate, pH 8.3, 2 mM EDTA, 20% acrylamide, and 8 M urea). Gels were dried, exposed in a PhosphorImager cassette, and analyzed using a Typhoon 8610 Variable Mode Imager (Amersham Biosciences) and quantitated using ImageQuant 5.2. Percent inhibition (%*I*) was calculated using the following equation:

$$\%I = 100 \times [1 - (D - C)/(N - C)]$$

where *C*, *N*, and *D* are the fractions of 21-mer substrate converted to 19-mer (3′-end processing product) or strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. The IC_{50} values were determined by plotting the logarithm of drug concentration versus percent inhibition to obtain concentration that produced 50% inhibition.

4.7.4. Anti-HIV-1 activity assay. The testing of the ability of potent compounds to inhibit HIV replication in cell culture was done according to a previously reported procedure.[38] PBMC (10^7 cells/T25flask) were stimulated with phytohemagglutinin for 3 days and infected with a wild-type HIV-1 strain (strain LAI) at 100 50% tissue culture infective doses, as described previously.[38] The cultures were kept for 5 days in the presence of test compounds at serial 1-log dilutions. Subsequently, human PBMC were removed from the culture supernatant by centrifugation (400*g*, 10 min, 4 °C). This clarified supernatant was tested by a reverse transcriptase assay.

4.7.5. Cytotoxicity assays. The cytotoxicity of compounds was evaluated using un-infected PBMC and CEM leukemia cells, as well as African green monkey kidney cells, Vero, according to a previous method.[39] PBMC were obtained from whole blood of healthy individuals, while CEM and Vero cells were obtained from the American Type Tissue Collection (Rockville, MD). The PBMC and CEM cells were cultured in the presence or absence of compound for 6 days. After this time period, cells were stained with Trypan blue dye, and counted for cell proliferation and viability according to the previously reported procedure.[40] Only the effects on cell growth are reported, since they correlated well with cell viability. For Vero cells the incubation period was 3 days, and toxicity was evaluated by viable cell counting using a hemacytometer as described previously.[41]

4.8. Molecular docking

Monomer B of the crystal structure of HIV-integrase with PDB ID of 1BIS[36] was used for docking, since it has the flexible catalytic loop resolved, which is missing in the IN core structure in complex with the inhibitor 5-CITEP (PDB code 1QS4).[42] The Mg^{2+} ion was placed in the active site between the carboxylate groups of catalytic amino acid residues Asp64 and Asp116 using the geometry of the Mg^{2+} ion present in subunit A of the 1QS4 structure[42] as reference. Phenanthridinones were constructed using the Biopolymer module in the Sybyl program (version 7.0, Tripos Associates Inc, St. Louis, MO). The β-diketo moiety was constructed as the keto-enol tautomer for docking. The program GOLD (v2.0)[35] from Cambridge Crystallographic Data Center, UK, was used to dock the ligands into the active site. GOLD is an automated ligand-docking program that uses a genetic algorithm to explore ligand conformational flexibility. It also performs limited exploration of receptor flexibility by optimizing torsion angles of serine and threonine hydroxyls, and lysine NH_3+ groups. The active site was defined as residues lying within 15 Å radius of the active Mg^{2+} ion. Ten different docking solutions were obtained for each molecule, the docking being terminated if the top ten solutions were within an RMSD of $\leqslant$1.5.

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