Synthesis, DNA Binding, Docking and Photoclevage Studies of Novel Benzo[*b*][1,8]naphthyridines

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Abstract: The synthesis and docking studies of novel benzo[*b*][1,8]naphthyridines are described**.** The docking studies show that the derivatives prefer to bind the AT-rich region of double stranded DNA (ds-DNA). The maximum binding energy -7.16 (kcal/mol) was observed for benzo[*b*][1,8]naphthyridine-5-thiol **5a** and it is a better candidate as an enantioselective binder to ds-DNA than the other derivatives of benzo[*b*][1,8]naphthyridines. When photoirradiated at 365 nm, benzo[1,8]-naphthyridines have been found to promote the photocleavage of plasmid pUC19 DNA.

Key Words: Synthesis, DNA minor groove, Photoclevage, Docking studies, Benzo[*b*][1,8]naphthyridines.

1. INTRODUCTION

DNA represents one of the most important molecular cellular targets of several chemotherapic drugs. Molecular recognition of DNA by small molecules and proteins is a fundamental problem in drug design. Polycyclic heterocycles having a planar structure can be effective pharmacophore moieties of DNA-interactive drugs because they can insert between the stacked base paired oligonucleotides. Moreover, if they bear suitable side chains, the interactions of polyheterocycles with the other important architectural feature of DNA and its minor groove would improve the latter's effect. Thus, the minor groove of DNA is the target for a wide range of anticancer, antiviral and antiprotozoal agents. Noncovalently binding molecules tend to bind to the minor groove of AT-rich sequences [1], and several are of current clinical use [2]. They are preferentially taken up, often by active transport mechanisms, into susceptible cell types, and exert their effect by blocking topoisomerases, and sometimes by acting as global inhibitors of transcription. Relatively little is understood at present about the mode of action at the molecular level of the majority of minor groove-interacting drugs, although there is increasing evidence that they may act by directly blocking or inhibiting protein–DNA recognition [3].

On the other hand, Photodynamic therapy (PDT) is an emerging method of noninvasive treatment of cancer in which drugs like Photofrin shows localized toxicity on photoactivation at the tumor cells leaving the healthy cells unaffected [4]. Along these lines, there has been increased interest in the discovery and investigation of compounds that damage DNA upon irradiation. These compounds, also called photonucleases [5], exhibit a large potential for therapeutical applications such as photodynamic chemotherapy [6], because they are often inert until activated by light and, thus, the DNA-damage reaction may be controlled both in a spatial and temporal sense. Photonucleases, like any other small DNA-binding molecules, associate by intercalation or by fitting into the minor groove [5,6]. Importantly, the type and the efficiency of the photocleavage reaction will depend on the binding affinity and the binding site that the photonuclease occupies.

There has been very little application of docking studies in interpretation of the DNA binding affinity, in modern chemistry and biochemistry. To obtain a significant correlation, it is essential that appropriate descriptors are employed, whether they are theoretical, empirical or derived from readily available experimental characteristics of structures. Many descriptors reflect simple molecular properties and can thus provide insight into the physicochemical nature of the activity/ property under consideration [7]. Subsequently, several researchers have reported correlations for a wide variety of chemical properties including DNA binding studies [8,9].

Further, 1,8-Naphthyridine derivatives have attracted considerable attention because, their skeleton is present in many compounds that have been isolated from natural substances, with various biological activities. Nalidixic acid, for example, possesses strong antibacterial activity and used mainly for the treatment of urinary tract infections with gram negative pathogens [10]. In addition, gemifloxacin is antibacterial [11], is used as an anesthetic [12] and is also employed for treatment of Alzheimer's disease [13]. 2-Amino-*N*-hydroxy-1,8-naphthyridine-3-carboxamidine possesses herbicidal properties and used for the selective control of weeds in barley, wheat, maize, sorghum and rice crops [14]. Indeed, some 3-phenyl-1,8-naphthyridines have been reported to show significant activity as inhibitors of human platelets aggregation induced by arachidonate and collagen

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[15]. 4-(*N*-methylenecycloalkylamino)-1,8-naphthyridine derivatives are effective as antihypertensive agents [16]. 7-Amino-2-(4-carbethoxypiperazin-1-yl)-4-phenyl-1,8-naphthyridine has recently been synthesized and reported to have marked activity against mycobacterium tuberculosis [17].

Hence, in view of the biological importance of 1,8-naphthyridines and in continuation of our interest in their synthesis [18,19], herein we wish to report a simple, eco-friendly synthesis of substituted benzo[*b*][1,8]naphthyridines, their DNA binding, docking and photocleavage studies.

2. RESULTS AND DISCUSSION

In our attempts to search for novel antitumor agents, we extended the interest to naphthyridine heterocycles and synthesized benzo[*b*][1,8]naphthyridine derivatives with the aim of evaluating their DNA binding and photonuclease studies. We have synthesized series of benzo[*b*][1,8]naphthyridines (Scheme **1**) using a simple, efficient and scientifically based framework for greener preparation of these quinolines in a manner that renders the materials less mobile in the environment and reduces or eliminates the use and generation of hazardous substances. For each of the compounds with three different derivatives containing H, I, CH_3 substituents were prepared and their DNA binding and docking studies were performed for comparison. The results reveal that, the unsbstituted benzo[*b*][1,8]naphthyridines, **3a**, **4a** and **5a** showed remarkable activity compared to substituted compounds of the particular series. In addition, we studied the photocleavage activity of unsbstituted benzo[*b*][1,8] naphthyridines based on the remarkable results obtained by DNA binding and docking studies.

2.1. Chemistry

The synthesis of the benzo[*b*][1,8]naphthyridin-5(5a*H*)-one **3a** was achieved by coupling 2-aminopyridine and 2-chlorobenzoic acid in the presence of polyphosphoric acid. The compounds were characterized by elemental analysis, IR, ^{1}H NMR and mass spectra. As an example, the I.R spectra of compound **3a** showed the presence of a peak at 1710 cm^{-1} corresponding to the stretching frequency of carbonyl group. The ^{1}H NMR spectra of compound **3a** showed the multiplet in the region δ 6.02-8.80 ppm. The structure was

also confirmed by mass spectra which showed the molecular ion peak at m/z=**197**[M+H]$^+$. Compound **3a** was halogenated using phosphorus oxychloride (POCl$_3$) in an aqueous medium to obtain compound **4a**. The infrared spectra of the compound **4a** showed the absence of carbonyl stretching frequency at 1710 cm^{-1}.

Benzo[*b*][1,8]naphthyridine-5-thiol was obtained by refluxing 5-chlorobenzo[*b*][1,8]naphthyridine in DMF in the presence of sodium sulfide flakes. The I.R spectra of compound **5a** showed the presence of a peak at 2630 cm^{-1} corresponding to the stretching frequency of >C=S or >C-SH group. The ^{1}H NMR spectra confirmed the presence of the mercapto group with a signal at δ 11.3 (1H). The molecular ion peak show at m/z=213[M+H]$^+$.

2.2. DNA Binding by Absorption Spectral Studies

DNA-binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA [20]. A variety of small molecules interact reversibly with double-stranded DNA, primarily through three modes: (i) electrostatic interactions with the negative charged nucleic sugar–phosphate structure, which are along the external DNA double helix and do not possess selectivity; (ii) binding interactions with two grooves of DNA double helix; and (iii) intercalation between the stacked base pairs of native DNA.

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques. Binding of benzonaphthyridines with DNA results in hypochromism and bathochromism shift in absorption spectra, this may be due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore of benzo[*b*][1,8]naphthyridines and the base pairs of DNA. The extent of the hypochromism commonly parallels the intercalative binding strength. The absorption spectra of **3a**, **4a** and **5a** in the absence and presence of ct-DNA are given in Fig. (**1**). The absorption results have shown that, as the concentration of DNA is increased from 10 μm to 50 μm the absorption bands of benzonaphthyridine **3a** at 305 nm, **4a** at 285 nm and **5a** at 278 nm exhibited hypochromism at about 20.8, 23.6 and 26.3%, respectively.
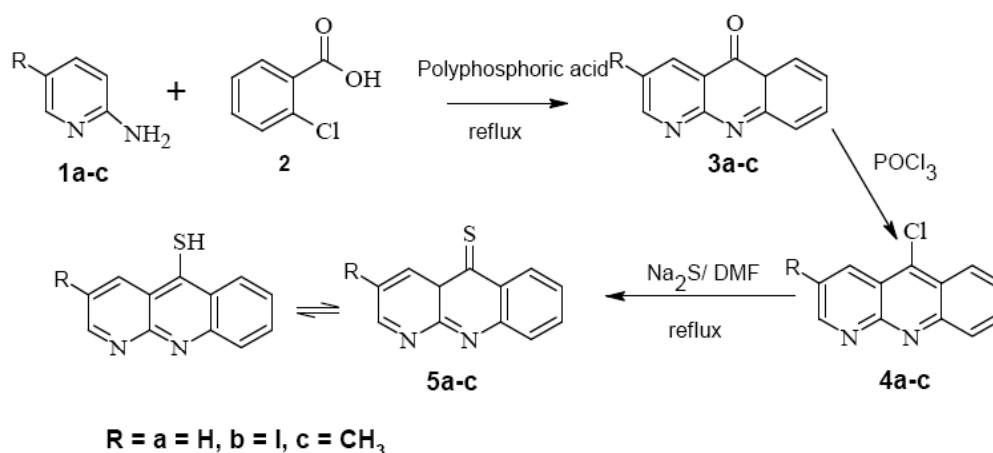
Scheme 1.
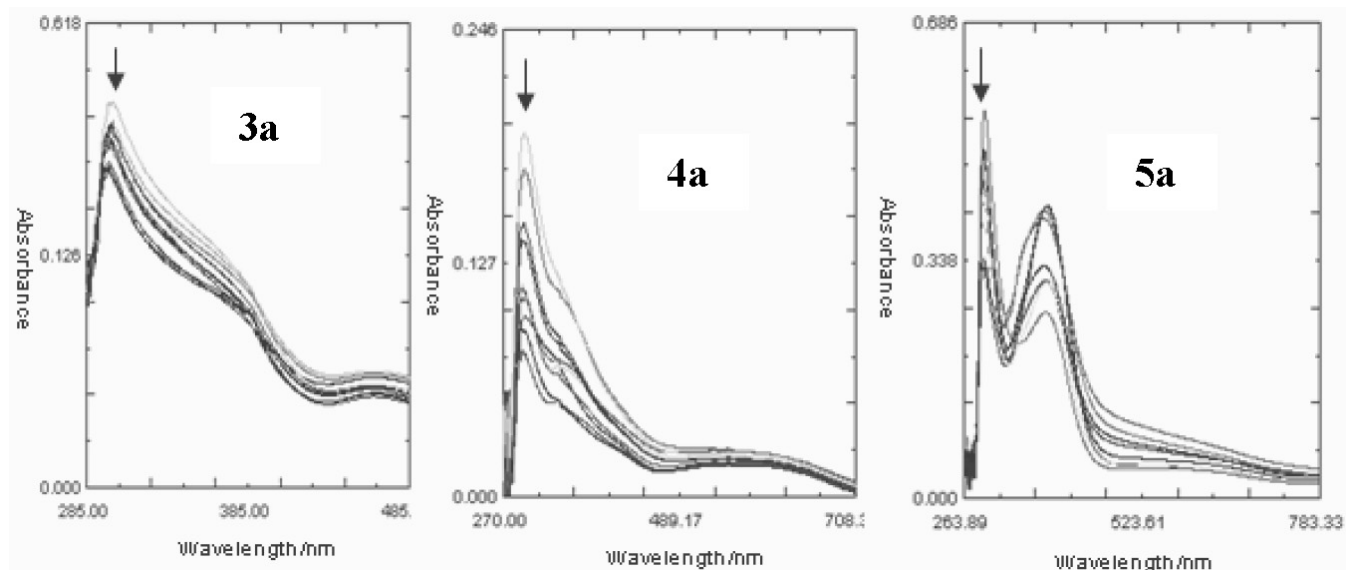
Fig. (1). UV absorption spectra of **3a**, **4a** and **5a** before irradiation in Tris-HCl buffer upon addition of calf thymus (ds) DNA.**3a**; control [DNA] = 0.5 ∞M [---], [**3a**] + [DNA] =10 ∞M [---]; 20 ∞M[---]; 30 ∞M[---]; 40 ∞M[---]; 50 ∞M[---], **4a**; control [DNA] = 0.5 ∞M[---], [**4a**] + [DNA] =10 ∞M[---]; 20 ∞M[---]; 30 ∞M[---]; 40 ∞M[---]; 50 ∞M[---] and **5a**; control [DNA] = 0.5 ∞M[---], [**5a**] + [DNA] =10 ∞M[---]; 20 ∞M[---]; 30 ∞M[---]; 40 ∞M[---]; 50 ∞M[---] DNA respectively. Arrow shows the absorbance changing upon the increase of DNA concentration.

In order to compare quantitatively the binding strength of these benzonaphthyridines, the intrinsic binding constants K_b of benzo[*b*][1,8]naphthyridines with DNA were calculated and presented in Table **1**. The binding constant (K_b) values obtained are 2.8 x 10^5 M^{-1} for **3a**, 2.5 x 10^5 M^{-1} for **4a**, 1.9 x 10^5 M^{-1} for **5a**, respectively.
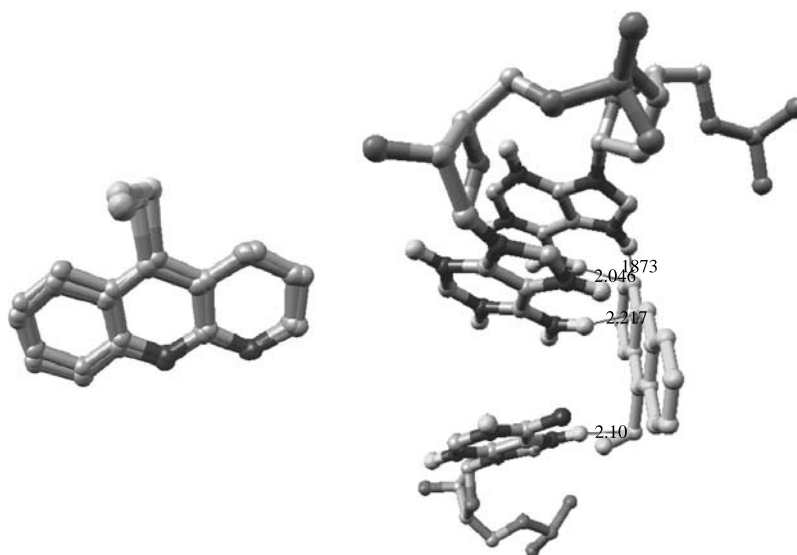
2.3. Docking

The docking studies were performed for benzo[1,8]naphthyridines considering the results in the past as that several aromatic compounds with related structures have DNA bind-

ing activity [21]. The intercalating interaction of docking virtual screening results of **5a** with ds-DNA as seen in Fig. (**2**). The VSA.DON (the sum of the Van der waals surface area of pure hydrogen bond donors as opposed to donor/acceptors) was also a high positive contributor in docking models. This suggests that hydrogen-bond donor groups enhance the DNA-binding ability of benzo[*b*][1,8]naphthyridine derivatives. It is significant that the docking was predictive and is indeed able to capture the appropriate physics of the benzonaphthyridines-DNA-binding process, thus affirming the validity of this modeling approach.

Table 1. The Data of Binding, Docking Energy, Inhibition Constants and DNA Binding Constant by Docking and Absorption Spectral Study

| Compound	Binding Energy (kcal/mol)	Docking Energy (kcal/mol)	Inhibition Constant	DNA Binding Constant (K_b)	λ_{max} (nm)	$\Delta\varepsilon$ (%)
3a	-6.86	-6.89	9.43 x e^{-6}	2.8 x 10^5 M^{-1}	305	20.8
3b	-6.48	-6.53	10.42 x e^{-6}	2.9 x 10^5 M^{-1}	317	19.6
3c	-6.62	-6.67	10.15 x e^{-6}	3.2 x 10^5 M^{-1}	312	20.1
4a	-7.01	-7.01	7.29 x e^{-6}	2.5 x 10^5 M^{-1}	285	23.6
4b	-6.97	-6.95	9.64 x e^{-6}	2.8 x 10^5 M^{-1}	310	20.4
4c	-6.85	-6.89	8.88 x e^{-6}	2.9 x 10^5 M^{-1}	296	21.3
5a	-7.16	-7.16	5.69 x e^{-6}	1.9 x 10^5 M^{-1}	278	26.3
5b	-6.93	-6.95	7.81 x e^{-6}	2.1 x 10^5 M^{-1}	309	22.7
5c	-7.08	-7.10	6.54 x e^{-6}	2.3 x 10^5 M^{-1}	288	25.2

Fig. (2). (**a**)Super imposition of compound **5a**. (**b**) Interaction of DNA basepairs with **5a**.

The docking studies results that all the tested compounds bind to ds-DNA. This could be due to the fact that they have an aromatic ring and the substituted oxy/chloro/sulfur group at C^5 of benzonaphthyridines. However, change in the affinity between the ligands and the DNA is due to the several functional groups that modify the electronic density on the aromatic ring and the oxy/chloro/sulfur atom. Comparative docking of ds-DNA with the candidate molecules benzo[*b*][1,8]naphthyridine derivatives yielded 10 best possible conformations with parameters including the least binding energy, docked energy, inhibition constant, RMSD, internal energy and torsional energy. Off all the three, highest affinity was observed for the derivative **3a, 4a** and **5a** which were showed -6.86, -7.01 and -7.16, kcal/mol binding energy with estimated inhibition constants of 9.43 x e^{-6}, 7.29 x e^{-6} and 5.69 x e^{-6} **,** and therefore these could be expected to form the more stable complex with ds-DNA (Table **1**). The Fig. (**3**) shows super imposition of the compound **3a, 4a** and **5a** which are targeted in between the d(CGCGAATTCGCG) base pairs of ds-DNA. The results show that benzonaphthy-

rines preferentially bind at the AT region of the dodecamer. This finding is consistent with the binding parameters obtained from the spectroscopic titration. The optimal binding conformation in the AT stretches of minor groove forms an extensive H-bonding network with the A and T base pairs of ds-DNA. Given that the substituent-effects play an important role in the electronic effects, the compound having more electrons donating groups in **3a, 4a** and **5a** had more binding affinity and it establishes that the fused linear aromatic structure of the molecule containing different subtituents are important to bind efficiently with the DNA in the minor groove.

2.4. Photonuclease Studies

The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoil form (Form I). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower-moving open circular form (Form II).
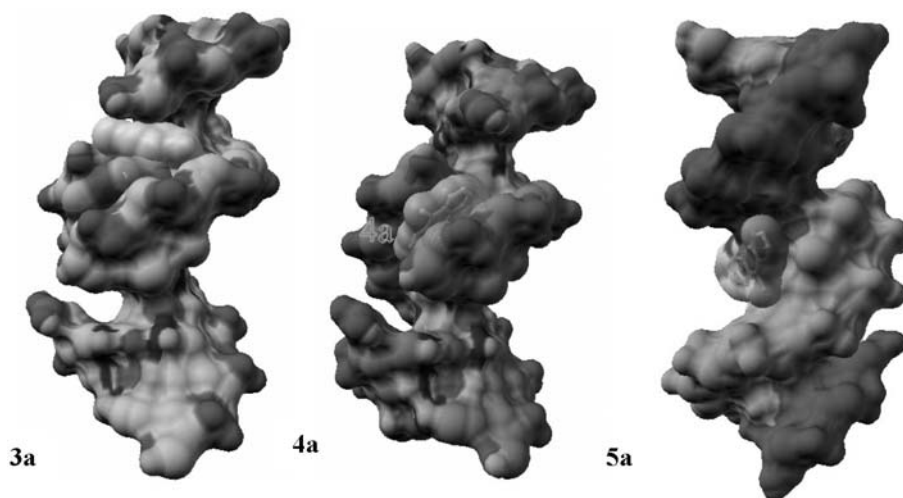
Fig. (3). View of energy minimized docked structures of 3a, 4a and 5a bound to d(CGCGAATTCGCG) [NDB Code: GDLB05].

Fig. (**4a**) shows gel electrophoresis separation of pUC19 DNA after incubation with different concentration of **3a**, **4a** and **5a** and irradiated for 2h, in 1:9 DMSO: Trisbuffer (20 μM, pH- 7.2) at 365 nm. DNA cleavage was not observed for controls in which benzonaphthytridines were absent (lane 1). With increasing concentration of 40 μM and 60 μM of **3a**, **4a** and **5a** (lanes 2–3, lanes 4–5 and lanes 6–7), the amount of Form I of pUC19 DNA diminish gradually, whereas Form II increases. Under comparable experimental conditions, compound **5a** exhibits more effective DNA cleavage activity than **3a** and **4a**. Further studies in detail are currently underway to clarify the cleavage mechanism.

3. EXPERIMENTAL SECTION

3.1. Materials

All chemicals used were of analytical reagent grade and purchased commercially and used without further purification. Aminopyridine derivatives were purchased from fine chemicals, INDIA. Calf thymus DNA and Plasmid DNA pUC19 were purchased from Bangalore Gene, Bangalore, INDIA. Tris-HCl buffer was purchased from Qualigens (INDIA). Benzonaphthyridines were synthesized with an efficient coupling of 2-aminopyridine and 2-chlorobenzoic acid and their structures were characterized by MS, elemental analysis, FTIR and ^{1}H NMR.

3.2. Methods

### 3.2.1. Synthesis of Benzo[b][1,8]naphthyridin-5(5aH)-one (3a)

A mixture of 2-aminopyridine (1 mmol) and 2-chlorobenzoic acid (1 mmol) and catalytic amount of polyphosporic acid (PPA) were taken in a round bottom flask containing ethanol. The reaction mixture was refluxed at 80-90 °C for 3-4 hrs. The completion of the reaction was checked by TLC, eluting the phase ethyl acetate: carbon tetrachloride and poured in ice-cold water. The solid separated was filtered, dried, and recrystallized from methanol to gave benzo[*b*][1,8]naphthyridin-5(5a*H*)-one (**3a**). The same procedure was used for the synthesis of other compounds (**3b-c**).

Benzo[b][1,8]naphthyridin-5(5aH)-one (3a)

White solid, Yeild: 80-85%, M.p. 161-164 °C. IR (KBr): 1710 cm^{-1} (C=O); ^{1}H NMR (DMSO d$_6$): 6.02 (d, 1H, *J*=7.2), 6.50 (d, 1H, *J*=7.2), 6.86 (d, 1H, *J*=7.5), 7.36 (d, 1H, *J*=7.5), 7.47 (d, 1H, *J*=8.0), 7.70 (d, 1H, *J*=8.0), 8.5 (d, 1H, *J*=8.6), 8.8 (d, 1H, *J*=8.6); Anal. Calcd (%) for C$_{12}$H$_8$N$_2$O: C; 73.46,
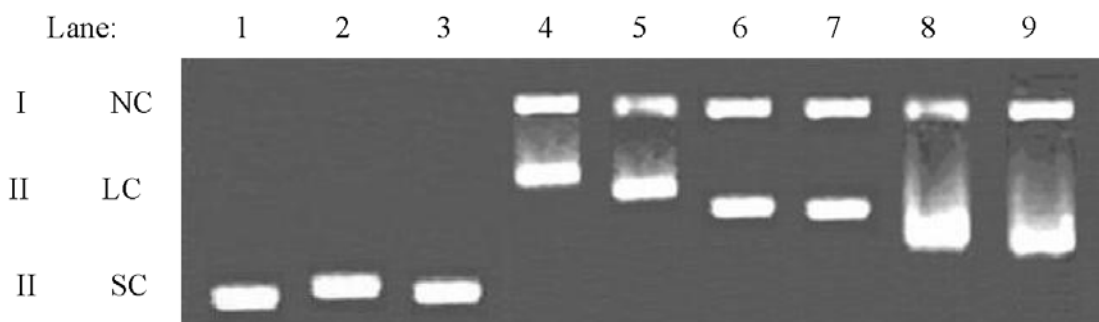
Fig. (4a). Light-induced cleavage of DNA by benzo[*b*][1,8]naphthyridine. Supercoiled DNA runs at position II (SC), linear DNA at position II (NC) and nicked DNA at position I (NC). Unless otherwise indicated, reactions in experiments **3a**, **4a** and **5a** were irradiated with UV light at 365 nm. Lane; 1: control, Lane; 2: **3a** without DNA, Lane; 3: **5a** without DNA, Lane; 4: 40μM (**3a**), Lane; 5: 60μM (**3a**), Lane; 6: 40μM (**4a**), Lane; 7: 40μM (**4a**), Lane; 8: 40μM (**5a**), Lane; 9: 60μM (**5a**).
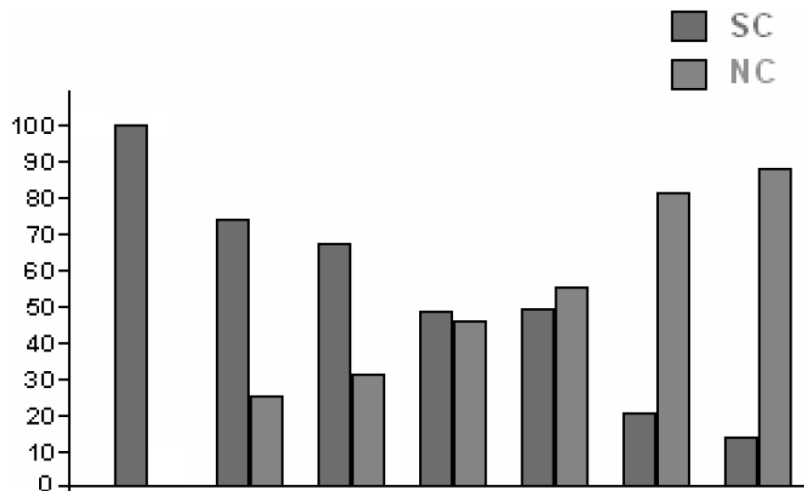
Fig. (4b). DNA supercoiled (SC) cleavage of pUC19 DNA upon irradiation in the presence of benzo[*b*][1,8]naphthyridines 3a, 4a and 5a. The sum of intensities of both bands is standardized to 100% for each individual lane.

H; 4.11, N; 14.28. Found: C; 73.44, H; 4.08, N; 14.27. mass m/z (M.+): 196.

3-Iodobenzo[b][1,8]naphthyridin-5(5aH)-one (3b)

White solid, M.p. Yeild: 75-80%, 168-170 $^{\circ}$C. IR (KBr): 1705 cm^{-1} (C=O); ^{1}H NMR (DMSO d$_6$): 6.21 (d, 1H, J=7.3), 6.36 (d, 1H, J=7.3), 6.55 (d, 1H, J=7.5), 7.20 (d, 1H, J=7.5), 7.33 (d, 1H, J=8.0), 7.61 (d, 1H, J=8.0), 7.84 (d, 1H, J=8.4), 8.00 (d, 1H, J=8.4); Anal. Calcd (%) for C$_{12}$H$_7$IN$_2$O: C; 44.75, H; 2.19, N; 8.70. Found: C; 44.73, H; 2.17, N; 8.68. mass m/z (M.+): 322.

3-Methylbenzo[b][1,8]naphthyridin-5(5aH)-one (3c)

White solid, Yeild: 75-80%, M.p. 142-145 $^{\circ}$C. IR (KBr): 1668 cm^{-1} (C=O); ^{1}H NMR (DMSO d$_6$): 6.15 (d, 1H, J=7.0), 6.28 (d, 1H, J=7.0), 6.42 (d, 1H, J=7.3), 6.80 (d, 1H, J=7.3), 7.26 (d, 1H, J=7.8), 7.46 (d, 1H, J=7.8), 7.80 (d, 1H, J=8.2), 7.95 (d, 1H, J=8.2); Anal. Calcd (%) for C$_{13}$H$_{10}$N$_2$O: C; 74.27, H; 4.79, N; 13.33. Found: C; 74.26, H; 4.77, N; 13.32. mass m/z (M.+): 210.

3.2.2. Synthesis of 5-chlorobenzo[b][1,8]naphthyridine

Benzo[*b*][1,8]naphthyridin-5(5a*H*)-one (1 mmol) and dry POCl$_3$ were taken in a round bottom flask. The reaction mixture was refluxed for just 1 hr. The complete reaction mixture was poured in to crushed ice. The product was extracted with pet ether and recrystalised with methanol to gave 5-chlorobenzo[*b*][1,8]naphthyridine (**4a**). The same procedure was used for the synthesis of other compounds (**4b-c**).

5-Chlorobenzo[b][1,8]naphthyridine (4a)

White solid, Yeild: 80-85%, M.p. 152-155 $^{\circ}$C. IR (KBr): 1558 cm^{-1} (CN); ^{1}H NMR (DMSO d$_6$): 7.02 (d, 1H, J=8.0), 7.34 (d, 1H, J=8.0), 7.45 (d, 1H, J=8.3), 7.65 (d, 1H, J=8.3), 7.70 (d, 1H, J=8.5), 8.07 (d, 1H, J=8.5), 8.20 (d, 1H, J=8.6), 8.54 (d, 1H, J=8.6); Anal. Calcd (%) for C$_{12}$H$_7$ClN$_2$: C; 67.15, H; 3.29, N; 13.05. Found: C; 67.13, H; 3.27, N; 13.04. mass m/z (M.+): 214.

5-Chloro-3-iodobenzo[b][1,8]naphthyridine (4b)

White solid, Yeild: 70-75%, M.p. 158-161 $^{\circ}$C. IR (KBr): 1553 cm^{-1} (CN); ^{1}H NMR (DMSO d$_6$): 7.00 (d, 1H, J=8.0), 7.28 (d, 1H, J=8.0), 7.36 (d, 1H, J=8.3), 7.55 (d, 1H, J=8.3), 7.76 (d, 1H, J=8.5), 8.00 (d, 1H, J=8.5), 8.18 (d, 1H, J=8.6), 8.35 (d, 1H, J=8.6); Anal. Calcd (%) for C$_{12}$H$_6$ClIN$_2$: C; 42.32, H; 1.78, N; 8.23. Found: C; 42.30, H; 1.76, N; 8.20. mass m/z (M.+): 340.

5-Chloro-3-methylbenzo[b][1,8]naphthyridine (4c)

White solid, 70-75%, M.p. 135-138 $^{\circ}$C. IR (KBr): 1562 cm^{-1} (CN); ^{1}H NMR (DMSO d$_6$): 6.95 (d, 1H, J=8.0), 7.16 (d, 1H, J=8.0), 7.40 (d, 1H, J=8.3), 7.52 (d, 1H, J=8.3), 7.68 (d, 1H, J=8.5), 7.85 (d, 1H, J=8.5), 8.00 (d, 1H, J=8.6), 8.32 (d, 1H, J=8.6); Anal. Calcd (%) for C$_{13}$H$_9$ClN$_2$: C; 68.28, H; 3.97, N;12.25. Found: C; 68.27, H; 3.95, N;12.23. mass m/z (M.+): 228.

3.2.3. Synthesis of Benzo[b][1,8]naphthyridine-5-thiol

5-Chlorobenzo[*b*][1,8]naphthyridine (1 mmol) in dry DMF (5 ml), sodium sulphide (1.5 mmol, fused flakes) were taken in a round bottom flask. The reaction mixture was stir-ring constantly for 1.5-2 hrs. The completion of the reaction monitored by TLC, the reaction mixture was poured in to crushed ice and made acidic with acetic acid. The product was filtered and washed with excess water, dried and recrystalised with excess methanol (**5a**). The same procedure was used for the synthesis of other compounds (**4b-c**).

Benzo[b][1,8]naphthyridine-5-thiol (5a)

Yellow solid, 70-75%, M.p. 169-171 $^{\circ}$C. IR (KBr): 2630 cm^{-1} (>C-SH); 7.15 (d, 1H, J=8.0), 7.36 (d, 1H, J=8.0), 7.34 (d, 1H, J=8.2), 7.60 (d, 1H, J=8.2), 7.65 (d, 1H, J=8.5), 8.10 (d, 1H, J=8.5), 8.27 (d, 1H, J=8.6), 12.20 (d, 1H, J=8.6); Anal. Calcd (%) for C$_{12}$H$_8$N$_2$S: C; 67.90, H; 3.80, N; 13.20. Found: C; 67.88, H; 3.79, N; 13.18, S; 15.11. mass m/z (M.+): 213.

3-Iodobenzo[b][1,8]naphthyridine-5-thiol (5b)

Yellow solid, 75-80%, M.p. 176-179 $^{\circ}$C. IR (KBr): 2624 cm^{-1} (>C-SH); 7.08 (d, 1H, J=8.0), 7.25 (d, 1H, J=8.0), 7.30 (d, 1H, J=8.2), 7.48 (d, 1H, J=8.2), 7.55 (d, 1H, J=8.5), 7.86 (d, 1H, J=8.5), 8.00 (d, 1H, J=8.6), 11.05 (d, 1H, J=8.6); Anal. Calcd (%) for C$_{12}$H$_7$IN$_2$S: C; 42.62, H; 2.09, N; 8.28. Found: C; 42.60, H; 2.07, N; 8.26, S; 9.48. mass m/z (M.+): 338.

3-Methylbenzo[b][1,8]naphthyridine-5-thiol (5c)

Yellow solid, 70-75%, M.p. 154-157 $^{\circ}$C. IR (KBr): 2617 cm^{-1} (>C-SH); 7.12 (d, 1H, J=8.0), 7.34 (d, 1H, J=8.0), 7.42 (d, 1H, J=8.2), 7.54 (d, 1H, J=8.2), 7.72 (d, 1H, J=8.5), 8.00 (d, 1H, J=8.5), 8.17 (d, 1H, J=8.6), 11.83 (d, 1H, J=8.6); Anal. Calcd (%) for C$_{13}$H$_{10}$N$_2$S: C; 69.00, H;4.45, N; 12.38. Found: C; 68.98, H;4.44, N; 12.35, S; 14.17. mass m/z (M.+): 226.

3.2.4. Benzonaphtyridines–DNA Interaction Studies

Tris-HCl buffer (5mM Tris-HCl, 50mM Nacl, pH-7.2, Tris=Tris(hydroxymethyl) amino methane) solution was prepared using deionised double distilled water. CT DNA (50 mg) was dissolved in 10 mL of 0.1 mol NaCl solution and kept at 4 °C for a few days. A little above solution was took out and diluted to a certain volume. A_{260}/A_{280} were determined on UV–Vis spectra in a Shimadzu model UV spectrophotometer at room temperature using quartz cuvettes of 10 mm light-path. Aliquots of a concentrated DNA solution were added to a cuvette filled with benzonaphthyridiens solutions (12–25 mM) and thoroughly mixed. Extreme care was taken to ensure that optical reference solutions were prepared in an identical manner. The intrinsic binding constant K_b was caluculated.

3.2.5. DNA-Photocleavage Experiments

The photonuclease activity of benzonapthyridines was studied using supercoiled (SC) pUC 19 DNA (0.5 µL, 0.5 µg) to its nicked circular (NC) form by agarose gel electrophoresis in Tris-HCl buffer (50 mM, pH 7.2) containing NaCl (50 mM) and 1.5 mM of 3, 4 and 5 in 1:9 DMSO:Tris buffer (pH 7.2) were photoirradiated using monochromatic UV light. The sample was then incubated for 1 hour at 37 °C followed by addition to the loading buffer containing 25% bromophenolblue, 0.25% xylene cyanol, 30% glycerol (3 µL), and finally loaded on to 0.8% agarose gel containing 1.0

µg/mL ethidium bromide. Electrophoresis was carried out at 50 V for 2-3 hours in Tris-borate EDTA (TBE) buffer. Bands were visualized by UV light and photographed to determine the extent of DNA cleavage. Due corrections were made for the trace of NC DNA present in the SC DNA sample and for the low affinity of EB binding to SC DNA in comparison to the NC form. The wavelength used for the photo-induced DNA cleavage experiments was 365 nm.

3.2.6. Molecular Docking

The ligand molecules, 3, 3 and 4 were designed and the structures were analyzed by using ChemDraw Ultra 6.0 and it was subjected for geometrical optimization using MM2 and energy minimized by steepest gradient method in Chem3D ultra 6.0. The final selected conformation of ligands was tested for Lipinski's rule, drug toxicity and other properties through preADMET server. The small-molecule topology generator prodrug server automatically generates the coordinates for all the ligands [22]. Automated docking was used to determine the orientation of inhibitors bind to the ds-DNA. A genetic algorithm method, implemented in the program AutoDock 3.0, was employed [23]. The crystal structure of the B-DNA dodecamer, d(CGCGAATTCGCG)2 (NBD code GDLB05) was obtained from the protein data bank. The co-ordinates for the heteroatom including water and other small molecules were removed. This structure was later added with polar hydrogens and kollmann charges to remove non-intergral chargers. For docking calculations, GasteigereMarsili partial charges were assigned to the ligands and nonpolar hydrogen atoms were merged [24]. All torsions were allowed to rotate during docking. Lennard- Jones parameters 12-10 and 12-6, supplied with the program, were used for modeling H-bonds and van der Waals interactions, respectively. The distance-dependent dielectric permittivity of Mehler and Solmajer was used to calculate the electrostatic grid maps [25]. Random starting points, random orientation, and torsions were used for all ligands. The translation, quaternion, and torsion steps were taken from default values in Auto-Dock. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters. The number of docking runs was 50, the population in the genetic algorithm was 250, the number of energy evaluations was 100,000, and the maximum number of iterations 10,000.

CONCLUSIONS

In summary, three novel benzonaphthyridines have been synthesized and characterized. Spectroscopic studies and Molecular modeling show that all the derivatives bind to ds-DNA through intercalation. Among these, **5a** shows the high binding affinity with DNA. When benzonaphthyridines are irradiated at 365 nm shows an efficient photocleavers of the plasmid DNA. These benzonaphthyridines may be useful as DNA probing tool.

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