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Synthesis of 6,12-Methanobenzo[*d*]pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-ones and Study of Their Interaction with DNA and β-Lactoglobulin

Nayim Sepay,[a] Chayan Guha,[a] Sanhita Maity,[a] Asok K. Mallik*[a]

Abstract: An efficient synthesis of 6,12-methanobenzo[*d*]pyrano-[3,4-*g*][1,3]dioxocin-1(12*H*)-ones, a new class of 2,8-dioxabicyclo-[3.3.1]nonanes, starting from 2-hydroxychalcones or their analogues and 4-hydroxy-6-methyl-2*H*-pyran-2-one has been achieved by use of amberlyst-15, a sulfonated polystyrene resin, as a recyclable heterogeneous catalyst. The methodology involves a domino sequence of Michael addition and two-stage heterocyclisation. Among the synthesized products, two show significant ct-DNA-binding property and all show strong binding with the carrier protein β-lactoglobulin. Study of antibacterial property done on two bacterial species using four compounds showed that two of them are moderately active.

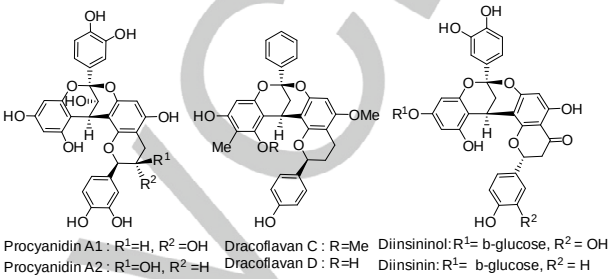


Figure 1. Several bioactive 2,8-dioxabicyclo[3.3.1]nonanes of natural origin.

Introduction

Compounds possessing a bicyclo[3.3.1]nonane framework with one or more heteroatom are found to be very important due to their cleft shaped structure.[1] A good number of natural products having a 2,8-dioxabicyclo[3.3.1]nonane framework in their structural motif have been reported to show interesting biological activities like antioxidant, anti-inflammatory, COX-2 enzyme inhibitory, antiviral, immunomodulatory and antitumor activities.[2] Several such compounds[2b,3] are shown in Figure 1. Again, compounds containing a 2-pyrone moiety are known to occur in different types of fungi[4] and they can be easily obtained by conventional organic synthesis[4a,b,5] as well as *in vitro* synthesis.[6] They are known to show antifungal, antibiotic, anticancer, cell toxin, neurotoxin and plant toxin activities.[4,6,7] 4-Hydroxy-6-methyl-2*H*-pyran-2-one (triacetic acid lactone) is the simplest natural product of the 2-pyrone family[4a,e] and available commercially. Other complex molecules containing either a fused or attached 2-pyrone moiety are also known as natural products.[5,7] Several bioactive 2-pyrone derivatives[7] are shown in Figure 2.

Being interested from the reported biological activities of natural 2,8-dioxabicyclo[3.3.1]nonanes, recently several research groups have developed synthetic routes to different compounds containing this framework.[8] On the other hand, development of synthetic routes to simple, attached and fused 2-pyrones is an interesting research area in organic chemistry even up to the present time.[4a,5,6] The common features of the synthetic routes to 2,8-dioxabicyclo[3.3.1]nonanes are Michael addition followed by two stage heterocyclisation. However, some of these methods suffer from drawbacks like use of expensive catalysts[8a] and application of harsh reaction condition like heating in a sealed tube.[8b] Recently, we studied the reaction of

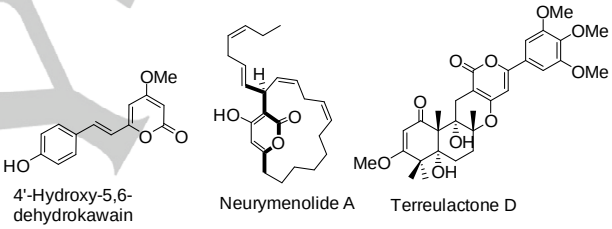
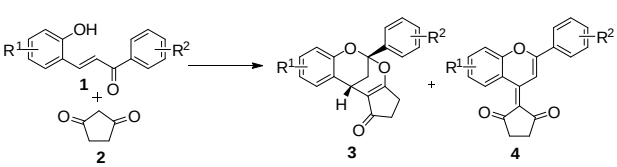


Figure 2. Several bioactive compounds containing a 2-pyrone moiety.

2-hydroxychalcones (**1**) and cyclopentane-1,3-dione (**2**) under amberlyst-15 catalyzed condition and observed that when the reaction was conducted in an aerial atmosphere it gave fused 2,8-dioxabicyclo[3.3.1]nonanes (**3**) as major product in a short reaction time (3-4 h) but 2-(2-phenyl-4*H*-chromen-4-ylidene)-cyclopentane-1,3-diones (**4**) in a longer reaction time (10-12 h). In an inert atmosphere, however, **3** was found to be the only product (Scheme 1).[9]

2,8-Dioxabicyclo[3.3.1]nonanes having V-shaped rigid geometry are structurally analogous to Tröger's base.[8b] Considering the importance of 2,8-dioxabicyclo[3.3.1]nonanes and 2-pyrones for their biological activities briefly mentioned above, ability of some Tröger's base derived bicyclo[3.3.1]-nonanes having cleft-like structure to bind with DNA[10] and



Scheme 1. Formation of 2,8-dioxabicyclo[3.3.1]nonanes in our previous study.

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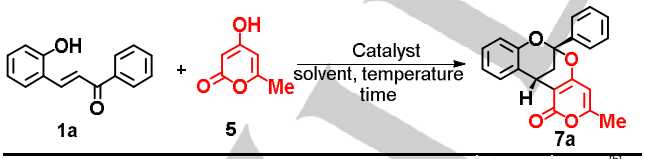
reported Michael donor property of 4-hydroxy-6-methyl-2*H*-pyran-2-one (**5**),[11] we set our objective to study the reaction of 2-hydroxychalcones (**1**) and their analogues (**6**) with **5** in boiling toluene under amberlyst-15 catalyzed condition with a view to synthesizing molecules incorporating both 2,8-dioxabicyclo-[3.3.1]nonane and 2-pyrone moieties in their structures. As an outcome of our endeavor, we could develop a simple and efficient methodology for synthesis of 6,12-methanobenzo[*d*]-pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)ones (**7** and **8**). Getting these new 2,8-dioxabicyclo[3.3.1]nonane derivatives in hand, we first undertook a study of their interaction with DNA. Out of a total of sixteen compounds of the series **7** and **8**, two showed significant DNA-binding property. β-Lactogobulin (β-lg) is a well-known carrier protein.[12] The current literature shows that there are a number of reports of its use for transport of several types of biologically active molecules.[12] Since the compounds of the series **7** and **8** are almost insoluble in water and some of them show significant DNA binding property, it was then our interest to investigate their interaction with β-lg. This investigation also produced some interesting results. Lastly, study of antibacterial property done on two bacterial species using four compounds showed that two of them are moderately active. Our findings on the above-said synthetic, binding and antibacterial studies are presented in this paper. It may be mentioned here that none of the recent papers dealing with synthesis of 2,8-dioxabicyclo-[3.3.1]nonane derivatives[8] reports any biophysical or antibacterial study of the synthesized compounds. Moreover, any independent study in the said areas with those compounds did not appear in the literature subsequently.

Results and Discussion

Synthesis of the New Compounds 7a-l and 8a-d

The present study started with the reaction of 2-hydroxychalcone (**1a**) and 4-hydroxy-6-methyl-2*H*-pyran-2-one (**5**) under amberlyst-15 catalyzed reaction condition. The initial success of the reaction to produce the 2,8-dioxabicyclo[3.3.1]-nonane derivative **7a** encouraged us to optimize the process by variation of the solvent, catalyst, amount of catalyst and reaction time under reflux condition. The results of the optimization study are given in Table 1. Under the optimized reaction conditions, eleven more compounds of the series **7** could be synthesized in good to very good yield by using different 2-hydroxychalcones (**1**); use of **6** instead of **1** gave four more structurally analogous compounds (**8**) (Table 2). All the synthesized compounds were new and they were characterized from their analytical and spectral data. In case of compound **7d** confirmation of the structure was done from single crystal X-ray crystallography

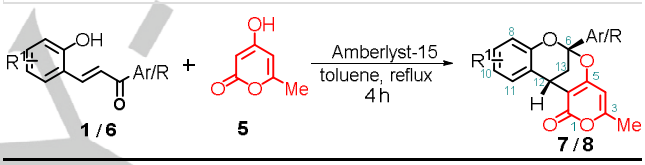
Table 1. Optimization of the reaction condition for synthesis of **7a**

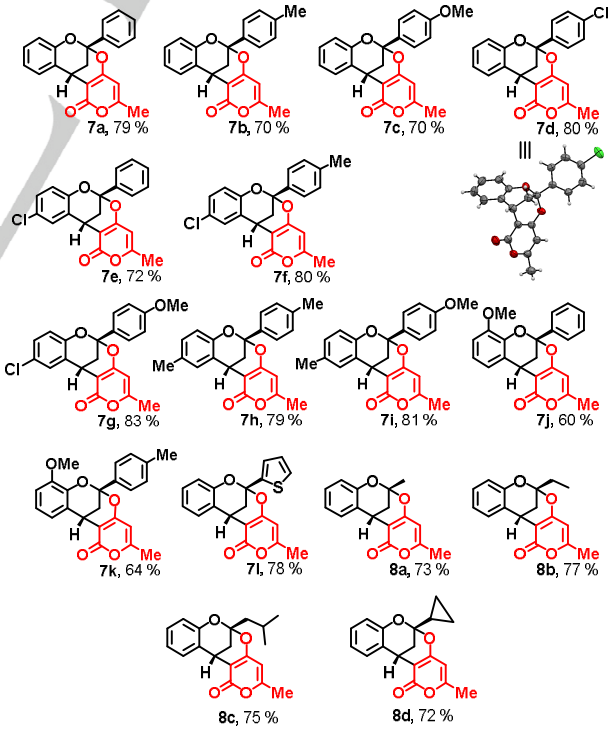


Entry	Solvent	Catalyst	Amt. of Catal.[a]	Temp. (°C)	Time (h)	Yield[b] (%)
1	Ethanol	-	-	78[c]	24	
2	Toluene	-	-	110[c]	24	12
3	Toluene	$FeCl_3$[d]	5	110	10	5
4	Toluene	$ZnCl_2$[e]	5	110	10	10
5	Toluene	PTSA	10	110	10	28
6	Toluene	Amb.-15	10	110	10	58
7	Toluene	Amb.-15	20	110	4	70
8	**Toluene**	**Amb.-15**	**30**	**110**	**4**	**79**
9	Toluene	Amb.-15	40	110	4	75
10	Ethanol	Amb.-15	50	110	4	32
11	DMF	Amb.-15	40	110	4	55
12	CH_3CN	Amb.-15	40	110	4	47

[a] For 1mmol of each of **1a** and **5**; [b] Isolated yield; [c] Reflux temp.; [d] Anhydrous; [e] Fused; Amb.-15 = Amberlyst-15

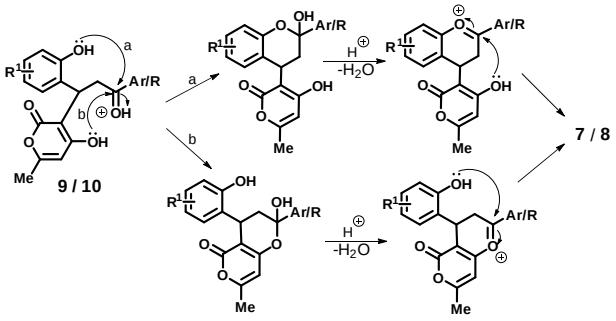
Table 2. Reaction of 2-hydroxychalcones and their analogues (**1**) with 4-hydroxy-6-methyl-2*H*-pyran-2-one (**5**)





(CCDC No. **CCDC 1548651**) also. Cleft-like geometry of the molecule is clearly evident from the ORTEP diagram (Table 2).

The mechanism of this transformation appears very simple. The first step is an acid catalyzed Michael addition involving **1** / **6** and **5**. The intermediates (**9** / **10)** thus formed undergoes two consecutive heterocyclisation processes in two possible routes leading to compounds **7** / **8** (Scheme 2).



Scheme 2. Proposed mechanism for the formation of **7** / **8.**

The heterogeneous catalyst was found to be recoverable after the reaction and can be recycled effectively up to the fifth cycle (Table 3).

Table 3. Recycling of the catalyst in the synthesis of **7a**

Entry	Condition of the catalyst	Time (h)	Yield (%)
1	Fresh	4	79
2	Run 1	4	75
3	Run 2	4	72
4	Run 3	4	69
5	Run 4	4	65

Study of Interaction with DNA

DNA is an important genetic bio-macromolecule and it regulates the structure and function of the cell. The mechanisms of action of drugs are related to a binding process, which generally involves an interaction of small drug molecules with bio-macromolecules, such as DNA, RNA or protein.[13] Furthermore, such binding can interfere with DNA replication and RNA transcription, providing valuable insight into how the drugs control the gene expression.[13,14] Understanding of the interaction mechanism between small molecules and DNA is a matter of paramount importance as this gives very useful information for designing new and more efficient therapeutic agents.[15] Since the synthesized compounds **7a-l** and **8a-d** possess a molecular geometry similar to Tröger's base derived bicyclo[3.3.1]nonanes which are known to show interesting DNA-binding property,[10] we got interested in undertaking a study of their binding with ct-DNA with the help of UV-vis and fluorescence spectroscopy. In this study, it was observed that two compounds (**7b** and **7c**) of the series showed significant binding with ct-DNA.

Study of Absorption Spectra

Small molecule-DNA interaction can be easily studied and illustrated using UV-vis spectroscopy, a simple and most commonly used technique.[10,15d,e] The study can be carried out by monitoring changes in absorption properties of either DNA or the small molecule. In aqueous 10 mM phosphate buffer solution, *i.e.*, at the physiological pH condition (pH = 7.4), each of the synthesized compound of the series **7** and **8** exhibited absorption peak near 300 nm along with a high energy absorption. There was considerable change in the absorbance of **7b** and **7c** near 230 nm and 340 nm upon binding to ct-DNA under the above mentioned condition as shown in Figures 3 (a) and (b). For both the compounds **7b** and **7c**, it was observed that on gradual addition of ct-DNA (0 to 70 μM) the absorption intensity decreased in the 200-220 nm region with formation of a hump near 220 nm and the same increased slightly around 340 nm, these were accompanied with *ca.* 8 nm bathochromic shift of the 300 nm peak. The results thus suggest a significant binding of these two compounds with ct-DNA.

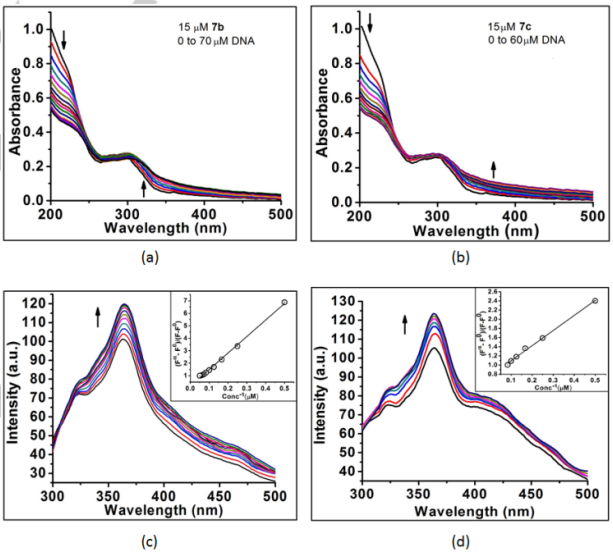


Figure 3. UV-visible spectral changes of (a) **7b** (15 μM) and (b) **7c** (15 μM) and fluorescence spectral changes of (c) **7b** (10 μM) and (d) **7c** (10 μM) with the gradual addition of ct-DNA at pH 7.4. The insets of fluorescence spectra shows the plot of $(F^{\alpha} - F^{0})/(F - F^{0})$ vs 1/[DNA].

Study of Fluorescence Spectra

In order to elucidate the interaction of the synthesized compounds **7b** and **7c** with ct-DNA, we studied their steady state fluorescence. We investigated the fluorescence spectra of both these compounds for the study as the endogenous fluorescence property of ct-DNA is very insignificant.[15d,e] The emission spectra of **7b** (upon excitation at 295 nm) in 10 mM phosphate buffer (pH 7.4) showed two maxima around 323 and 363 nm (Figure 3 (c)). A hyperchromism of fluorescence with a 2 nm red shift of the 363 nm peak was observed on addition of ct-

DNA, which collectively indicated a binding interaction of **7b** with ct-DNA. For having an insight of this interaction it was our endeavor to get Benesi-Hildebrand plot[16] which is a plot of $(F^{\alpha} - F^{0})/(F - F^{0})$ *vs* inverse of DNA concentration, where F^{α} and F^{0} are the fluorescence intensities in presence of large excess and absence of DNA, respectively, and F is the fluorescence intensities in presence of DNA at different concentrations. The linearity of this plot indicates a1:1 binding of the compound with DNA. Again, the plot of $(F^{\alpha} - F^{0})/(F - F^{0})$ *vs* $1/[DNA]^2$ shows a non-linear plot (provided in Supporting Information (SI)), which confirms a 1:1 binding interaction.[16] Similar results were obtained with **7c** also. Binding constants for the **7b**- and **7c**-DNA systems have been calculated from the slope of the linear plots and their values are 7.57×10^4 M^{-1} and 3.031×10^5 M^{-1}, respectively. These values suggest that the binding between each of **7b** and **7c** with ct-DNA is moderately strong.

Iodide Quenching Experiment

Negatively charged iodide ions, efficient fluorescence quencher for small fluorescent molecules in their aqueous solution, provide great help in understanding the mode of interaction between DNA and small molecules. As DNA containing a negatively charged phosphate framework can repel iodide ions, any small molecule bound to the DNA helix by intercalation is well protected to approach of anionic quenchers. The situation is different for molecules bound to DNA by electrostatic or groove binding as they are easily approachable by the said quenchers.[17] The binding mode of **7b** and **7c** to ct-DNA was investigated using fluorescence quenching of these compounds by KI as anionic quencher in the presence and absence of ct-DNA. The data obtained from the experiment were analyzed by calculating Stern-Volmer quenching constant Ksv in the Stern-Volmer equation.[18]

$$F^0/F = 1 + Ksv[Q] \qquad (1)$$

where F^0 and F denote the highest steady-state fluorescence intensity of the compounds in the absence and presence of the quencher (KI). The slope obtained from $[F^0/F]$ vs [Q] plot is the quenching constant Ksv. The binding mode of the small molecule can be understood from the values of Ksv obtained in absence and presence of DNA. Any unchanged Ksv in presence of DNA signifies electrostatic or groove binding interaction while a decrease of this constant under the same condition indicates intercalation.[15d,e] It is apparent from Figure 4 (a) that KI could effectively quench the fluorescence of **7b** both in absence and presence of ct-DNA in a buffer solution, and a Ksv value of 8.61 M^{-1} and 8.28 M^{-1}, respectively, was obtained. A similar experiment for **7c** gave analogous results (Figure 4 (b)) (without DNA Ksv = 16.7 M^{-1} and in presence of DNA Ksv = 15.1 M^{-1}). The slight difference of the Ksv values with and without DNA for both the compounds indicates groove binding for them.

Competitive Displacement Assay

Extensive applications of DNA binding dyes, whose binding mode to DNA is well established, are found for studying the mode of small molecule-DNA interaction. Competitive displacement of a bound dye from DNA helix by a small molecule indicates that the latter binds to the DNA in a similar fashion as the former.[19] Ethidium bromide (EB), a fluorescent DNA-intercalating dye, is often used to explore the binding mode of a molecule with DNA. To confirm the binding mode of each of **7b** and **7c** with DNA, we monitored the change of fluorescence peak at 618 nm of a preformed DNA-EB complex with successive addition of the compound. In this experiment it was observed that there was almost no change in intensity of the emission spectra (Figures 4(c) and 4(d)). This indicates that none of the compounds **7b** and **7c** is able to replace EB from the DNA-EB complex, thereby confirming that their binding is of non-intercalative type.

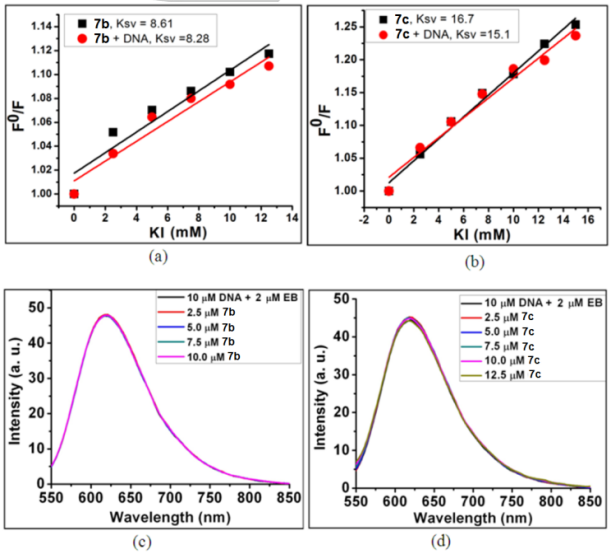


Figure 4. Plots of F^0/F versus [KI] at λ_{ex} 295 nm at pH 7.4 for (a) **7b** and (b) **7c**. Fluorescence spectral changes of DNA-EB (2 μM) at pH 7.4 with the gradual addition of (c) **7b** and (d) **7c**.

Molecular Docking Study

Molecular docking, a very effective theoretical tool, helps to predict the mode and site of binding at the target specific region of proteins, DNA etc.[12h,i] These macromolecules are generally amphiphilic in nature and interact with an amphiphilic organic small molecule through hydrogen bonding and weak forces like van der Waals forces, electrostatic effects and stacking interactions. Here, the binding of the compounds **7b** and **7c** with ct-DNA (PDB code 2BNA) was realized by utilizing molecular docking. The results of this study reveal that both the compounds bind at a groove of DNA through weak electrostatic

and/or π-staking interactions (Figure 5) as the molecules have large hydrophobic parts and aromatic rings (Figures 5(b) and 5(d)). The compound **7b** interacts with G4, A5, A6, T7 and G22 residues of DNA present at the groove. On the other hand, **7c** binds at the groove of DNA where D4, A5, T7 and C23 residues are in its vicinity. The first compound has few stacking interactions of which the interaction of the pyrone methyl group with A5 and A6 bases and that of the *p*-methylphenyl group with sugar hydrogens of DNA backbone are noteworthy. In case of **7c**, the *p*-methoxyphenyl group interacts through π-stacking with DNA bases G4 and A5. Its pyrone methyl and methoxy groups are also involved in other type of stacking interactions with DNA.

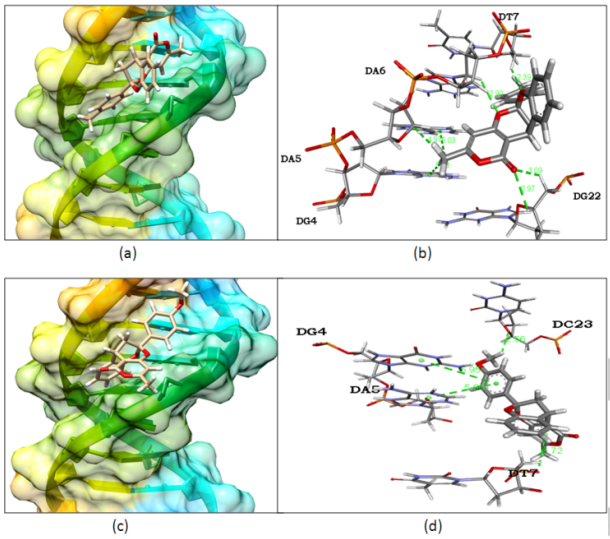


Figure 5. Docking poses (a) and (c) showing the groove binding of the compounds **7b** and **7c**, respectively, inside the ct-DNA. A close view of the binding pocket of DNA demonstrating the mode of interactions of **7b** and **7c** with some DNA bases, (b) and (d).

Study of Interaction with β-lg, a Carrier Protein

Carrier proteins play a key role in the transport of molecules across the cell boundary through active transport as well as facilitated diffusion, a type of passive transport.[20] These macromolecules have suitable pockets where they can bind small molecules which are transported by them.[16a,18] β-Lactoglobulin (β-lg) is one of the main lipocalin proteins of whey, the binding properties of which with varieties of organic molecules have been studied exhaustively.[12] Several important features such as its abundance from a cheap protein source, easy purification, possession of a number of natural nutrient binding sites, high water solubility and relative insensitivity to proteases at the acidic pH, make this globular protein a potential carrier for various small organic molecules.[12a,b,21] Thus, the current literature shows that this protein acts as carrier for a variety of bioactive small molecules such as folic acid,[12c] resveratrol,[12d,18] curcumin,[12d] fluorinated antibiotics like norfloxacin[12b] and ciprofloxacin,[12e] polyphenol flavanoids like genistein,[12d] quercetin,[12f] quercitrin[12f] and rutin[12f] and steroids and their derivatives[12b,g] etc. We observed that two compounds of the series **7** show significant binding with ct-DNA. As in the biological systems small organic molecules are carried to the cells by carrier proteins,[20] it was our interest to study the binding of the compounds of the series **7** and **8** with the carrier protein β-lg. The presence or absence of interaction was investigated using fluorescence in 5% aqueous ethanolic phosphate buffer (pH = 7.4). It was evident from the fluorescence study that all the compounds of the series **7** and **8** showed strong binding interaction, **7k** giving the best result.

Study of Fluorescence Spectra

The phenylalanine (Phe), tyrosine (Tyr) and two tryptophan (Trp-19 and Trp-61) amino acid residues of the protein β-lg are responsible for its intrinsic emission property. It shows emission spectrum with a maximum at 337 nm on excitation at 295 nm. The tryptophan residue Trp-19 located inside the hydrophobic environment of β-barrel, a core structure of β-lg consisting of eight anti-parallel beta-strands, contributes about 80% of the total fluorescence when the protein is excited at 295 nm. The second tryptophan residue (Trp-61) located at the hole of the barrel near the loop is “fluorescence silent” due to fluorescence quenching by a disulfide bond in its vicinity.[22] The fluorescence property of the protein often changes owing to protein–small molecule interactions. This is why the emission spectral techniques are widely used to investigate the binding ability of small molecules to the protein. Here, we investigated the effect of the compounds **7a-l** and **8a-d** on the intrinsic fluorescence property of β-lg through monitoring the change of emission spectra of the protein. It was observed that the emission intensity at 337 nm of β-lg decreases in the presence of increasing concentrations of **7a-l** and **8a-d** at pH 7.4 as shown in Figure 6. The observed decrease in fluorescence intensity may be ascribed to the binding of **7a-l** and **8a-d** with β-lg, because the intensity depends only on how much the Trp-19 residues of the protein is exposed by the change of solvent polarity or the presence of a specific quencher in its proximity. Therefore, it may be concluded that the compounds of the series **7** and **8** probably bind at a site near to the Trp-19.[23]

The fluorescence intensity of the protein was found to depend on the concentration of the small molecules **7a-l** and **8a-d**, which was analyzed using the Stern-Volmer plots. The plots of F_0/F *vs* concentration for each of **7a-l** and **8a-d** gave a straight line with an intercept as shown in the insets of the plots given in the SI file. Only four such plots are shown in Figures 6(a)-(d). The Stern-Volmer quenching constant Ksv at pH 7.4 for each of the compounds (Table 1, SI) indicated strong binding between β-lg and the compound. In this respect, the most strong binding was observed with **7k** (Ksv = 5.3×10^7 M^{-1}). The Ksv values for **7b** and **7c**, both able to bind with ct-DNA, were $3.9\times10^7 M^{-1}$ and 2.7×10^7 M^{-1}, respectively.

For this quenching, the binding sites (n) and the binding constant (K_S) can be calculated using the following equation[24]

$$\log [(F_0\text{-}F)/F] = \log K_s + n \log[Q] \quad (2)$$

where the parameters F_0, F and [Q] bear the same significance as in the Stern-Volmer equation. The linearity of the plot of log $[(F_0\text{-}F)/F]$ *vs* log [compound **7** / **8**] at pH 7.4 as shown in SI was found for all the compounds when the protein is excited at 295 nm. The number of binding sites (n) and K_s (binding constant) can be obtained from the slope and intercept, respectively, of the linear plot, which have been shown in Table 2 in SI. The values of n at pH 7.4 are very close to 1 for all the cases.

It may be mentioned here that the excitation wavelength of the protein and the compounds of the series **7** is same, *i.e.,* 295 nm, and their emission maxima are 337 and 364 nm, respectively (Figures 3(a)-(d) show the values for **7b** and **7c**). In all the cases, except for **7c**, **7i** and **7j**, only one emission peak was observed at 337 nm instead of the expected pair of peaks, which definitely indicates a strong binding interaction between the partners. It is interesting to note that with increasing concentration of the compounds **7c** and **7i** the intensity of the protein fluorescence at 337 nm decreases with the formation of a new peak at 393 nm (Figure 6), **7j** also gives analogous result (shown in SI). In these cases the new peak is far away from the corresponding peak of the compounds (at *ca.* 364 nm) and the peak saturates at their higher concentration. This interesting observation suggests the formation of an exciplex between β-lg and the compound at the excited state.[25] The stabilization associated with the formation of the exciplex may be assessed from the difference between the protein peak and the new peak which was found to be *ca.* 56 nm red shifted. This is indicative of the presence of hydrogen bonding interaction between **7c** / **7i** / **7j** and the protein.[25]

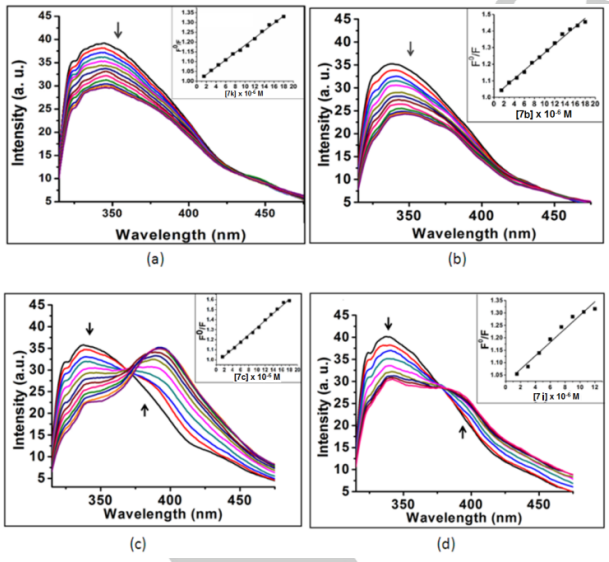


Figure 6. The changes of emission spectrum of β-lg (10 μM) with increasing concentration of the compounds (a) **7k**, (b) **7b**, (c) **7c** and (d) **7i**. The inset of each shows the plot of F^0/F versus [Concn.] at λ_{ex} 295 nm at pH 7.4.

Molecular Docking Study

The protein β-lg having 162 amino acid residues forms eight long anti-parallel β-strands to build a β-barrel, called calyx, and one α-helix at the outer surface of the barrel. This calyx is a hydrophobic pocket capable to provide a suitable place to small hydrophobic molecules inside it. There are also two more binding pockets at its outer surface, a channel between α-helix and the barrel and the region near Trp19–Arg124, which prefer binding with amphiphilic aromatic molecules through electrostatic and hydrogen bonding interactions.[21a]

Here, all the molecules of the series **7** / **8** are amphiphilic and bear aromatic ring in their structures. We performed docking study of the compounds **7k** and **7c** with β-lg (PDB code 1bsy) to find the binding pocket and the mode of interaction. As mentioned above, the compound **7k** shows highest binding affinity towards β-lg while **7c** forms exciplex with the protein. The molecule **7k** binds at a pocket 7.7 Å away from the Trp19 residue on the surface of the protein through various electrostatic interactions. Possibly, this binding changes the protein conformation in such a way that Trp19 moves inside the hydrophobic cavity. This may be the reason why the fluorescence quenching was observed on addition of **7k** and its analogues into the protein solution. The amino acid residues Lys101, Val123, Arg124, Glu127, Asp129 and Asp130 are inside the pocket (Figure 7 (a)). On the other hand, **7c** binds inside the calyx. In this case the distance between the molecule and Trp19 is about 15.5 Å. The molecule gets the amino acid residues Leu39, Val41, Lys60, Ile71, Ile84, Ala86 and Met107 around itself for electrostatic and π-stacking interactions and Lys69 for H-bonding interaction (Figure 7 (b)). These interactions may be involved in exciplex formation.

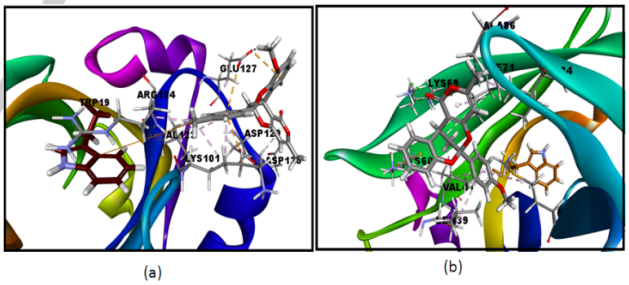


Figure 7. Docking poses showing the interaction sites of (a) the compound **7k** at the surface of β-lg and (b) the compound **7c** at the calyx of this protein.

Study of Antibacterial Properties

From the photophysical studies done on sixteen synthesized compounds it is evident that **7b** and **7c** show significant binding with ct-DNA and **7k** shows highest binding affinity towards β-lg. In the last part of the present work we undertook a study of antibacterial properties of these three compounds of the series **7** and of **8a** of the other series on one gram-positive (*Staphylococcus aureus*) and one gram-negative (*Escherichia coli*) bacteria. It was observed that among these

four compounds **7c** and **8a** showed moderate antibacterial properties towards both these bacteria (Figure 8).

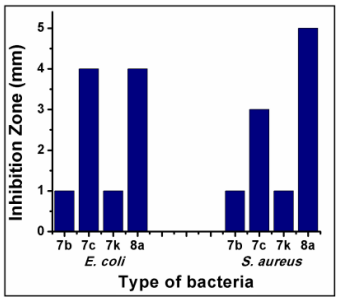


Figure 8 Results of antibacterial study done for **7b**, **7c**, **7k** and **8a** using well diffusion method in Nutrient Agar (NA) medium

Conclusions

Thus, we report here a facile synthesis of 6,12-methanobenzo[d]pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-ones, a new class of 2,8-dioxabicyclo[3.3.1]nonanes, using readily available starting materials by use of amberlyst-15, a sulfonated polystyrene resin, as a recyclable heterogeneous catalyst. The methodology involves a domino sequence of Michael addition and two-stage heterocyclisation. Among the sixteen synthesized products, two show significant ct-DNA-binding property and all show strong binding with the carrier protein β-lactoglobiulin. These interesting biophysical properties are indicative of the compounds having potential biological activities. Antibacterial property studied on two bacterial species (one gram-positive and one gram-negative) using four new compounds showed that two of them are moderately active.

Experimental Section

General

Standard literature procedures were followed to dry the solvents used in the experiments. Oven-dried round-bottomed flasks were used to perform the reactions. Thin-layer chromatography plates (Silica gel G) were visualized by exposure to ultraviolet light and/or iodine vapour. IR spectra were recorded on a Perkin Elmer FT-IR Spectrophotometer (Spectrum BX II) as KBr pellets.^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker 300 MHz NMR spectrometer. ^{1}H chemical shifts (δ) were reported in parts per million (ppm) taking $CHCl_3$ peak at δ 7.26 and ^{13}C chemical shifts taking the value for $CDCl_3$ at δ 77.0. Coupling constants (*J*) are quoted in hertz (Hz). HRMS were recorded on Waters Xevo G2-S QTOF and Waters Xevo G2QT mass spectrometers. Elemental analyses were done using two Perkin-Elmer 2400 Series II C, H, N analyzers. UV-vis and Fluorescence spectra were recorded on a SIMADZU UV1700 and an Agilent Technologies Cary Eclipse Fluorescence spectrophotometer, respectively.

Calf thymus DNA (ct-DNA) was purchased from Sigma Aldrich Corporation (MO, USA) and used without further purification. The homogeneous stock solutions of ct-DNA were prepared in 10 mM phosphate buffer (pH~7.4) by magnetically stirring for 24 h at 4^{0}C. The concentration of ct-DNA was estimated spectrophotometrically using the molar absorption coefficient, ε DNA = 6600 M^{-1} cm^{-1} at 260 nm. β-Lactoglobulin (β-lg) was isolated and purified from cow milk.[26] The final product was lyophilized and stored at 4 ^{0}C. Using the known extinction coefficient of β-lg (0.96 mg^{-1} mL^{-1} cm^{-1} at 280 nm) different concentrations of this protein were prepared by dissolving β-lg samples in milli-Q-water and then measuring the O.D. at 280 nm.

2-Hydroxychalcones and their analogues (**1**) required for this study were synthesized by base catalyzed condensation of salicylaldehydes and methyl ketones in ethanolic KOH and all of them were known compounds.[9,12h,27]

3.2 General Procedure for the Synthesis of 6,12-Methanobenzo-[d]pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-ones (7 / 8)

To a solution of a 2-hydroxychalcone or its analogue (**1** / **6**) (1 mmol) and 4-hydroxy-6-methyl-2*H*-pyran-2-one (**5**) (1 mmol) in anhydrous toluene (10 mL) was added amberlyst-15 (40 mg) at room temperature. The resulting mixture was refluxed with stirring for 4 h. After completion of the reaction, toluene was removed by distillation and dichloromethane was added to dissolve the crude product and then the catalyst was filtered off. The filtrate was concentrated and the resulting material was subjected to column chromatography over silica gel. The pure product thus obtained was finally crystallized from DCM-petroleum ether.

3-Methyl-6-phenyl-6,12-methanobenzo[*d*]pyrano[3,4-*g*][1,3]-dioxocin-1(12*H*)-one (7a): Colorless crystals (262 mg, 79% yield). M.p. 115-116 °C. IR (KBr): ν = 1710, 1652, 1591, 1481, 1457, 1441, 1400, 1339, 1319, 1242, 1141, 1116, 1019, 995, 865, 849, 832, 763, 698 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.69-7.65 (m, 2H, Ar-H), 7.50-7.45 (m, 4H, Ar-H), 7.22 (dt, *J* = 7.2 and 1.5 Hz, 1H, H-9), 7.04 (d, *J* = 8.1 Hz, 1H, H-8), 6.97 (t, *J* = 7.2 Hz, 1H, H-10), 5.93 (s, 1H, H-4), 4.23 (t, *J* = 2.7 Hz, 1H, H-12), 2.37 (dd, *J* = 14.4 and 3.0 Hz, 1H, H-13A), 2.29 (dd, *J* = 14.4 and 3.3 Hz, 1H, H-13B), 2.19 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.3, 162.9, 161.0, 151.4, 139.7, 129.3, 128.5, 128.2, 128.0, 125.6, 122.0, 116.3, 103.3, 100.0, 99.9, 32.8,

26.7, 19.9 ppm. HRMS (TOF): calcd. for $C_{21}H_{16}NaO_4$ $(M+Na)^+$ 355.0946; found 355.0933. Anal. calcd for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85. Found: C, 75.72; H, 4.96.

3-Methyl-6-(*p*-tolyl)-6,12-methanobenzo[*d*]pyrano-[3,4-*g*][1,3]dioxocin-1(12*H*)-one (**7b**)**:** Colorless crystals (242 mg, 70% yield). M.p. 118-119 °C. IR (KBr): ν = 1709, 1651, 1591, 1486, 1457, 1400, 1344, 1227, 1178, 1142, 1121, 1105, 1020, 1004, 992, 935, 891, 838, 814, 762 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.54 (d, *J* = 8.1 Hz, 2H, H-2′,6′), 7.48 (dd, *J* = 7.5 and 1.5 Hz, 1H, H-11), 7.27 (d, *J* = 8.1 Hz, 2H, H-3′,5′), 7.18 (dt, *J* = 7.5 and 1.6 Hz, 1H, H-9), 7.03 (br. d, *J* = 7.5 Hz, 1H, H-8), 6.96 (dt, *J* = 7.4 and 1.2 Hz, 1H, H-10), 5.92 (s, 1H, H-4), 4.21 (t, *J* = 2.7 Hz, 1H, H-12), 2.40 (s, 3H, 4′-CH_3), 2.40-2.28 (m, 2H, CH_2), 2.18 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.3, 162.9, 160.9, 151.4, 139.3, 136.8, 129.2, 128.1, 128.0, 125.6, 125.5, 121.9, 116.3, 103.3, 100.1, 99.9, 32.8, 26.7, 21.2, 19.9 ppm. HRMS (TOF): calcd. for $C_{22}H_{18}NaO_4$ $(M+Na)^+$ 369.1103; found 369.1072.

6-(4-Methoxyphenyl)-3-methyl-6,12-methanobenzo[*d*]-pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-one (7c): Colorless crystals (253 mg, 70 % yield). M.p. 127-128 °C. IR (KBr): ν = 1715, 1652, 1617, 1589, 1515, 1483, 1456, 1444, 1400, 1334, 1259, 1243, 1173, 1110, 1027, 999, 937, 882, 834, 814, 768 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 8.7 Hz, 2H, H-2′,6′), 7.48 (dd, *J* = 7.5 and 1.5 Hz, 1H, H-11), 7.18 (dt, *J* = 7.5 and 1.5 Hz, 1H, H-9), 7.02 (br. d, *J* = 7.5 Hz, 1H, H-8), 6.98-6.93 (m, 3H, Ar-H), 5.91 (s, 1H, H-4), 4.21 (t, *J* = 3.0 Hz, 1H, H-12), 3.86 (s, 3H, OCH_3), 2.39-2.18 (m, 2H, CH_2), 2.18 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.3, 162.9, 160.9, 160.3, 151.4, 131.9, 128.1, 128.0, 126.9, 125.6, 121.9, 116.2, 113.8, 103.3, 100.1, 99.9, 55.4, 32.9, 26.7, 19.9 ppm. HRMS (TOF): calcd. for $C_{22}H_{18}NaO_5$ $(M+Na)^+$ 385.1052; found 385.1039.

6-(4-Chlorophenyl)-3-methyl-6,12-methanobenzo[*d*]-pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-one (7d): Colorless crystals (293 mg, 80 % yield). M.p. 131-132 °C. IR (KBr): ν = 1718, 1654, 1590, 1487, 1447, 1399, 1339, 1244, 1176, 1136, 1112, 1088, 1016, 1000, 972, 937, 893, 793, 757 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.60 (d, *J* = 6.9 Hz, 2H, H-3′,5′), 7.48 (dd, *J* = 7.8 and 1.5 Hz, 1H, H-9), 7.43 (d, *J* = 8.4 Hz, 2H, H-2′,6′), 7.19 (dt, *J* = 8.9 and 1.5 Hz, 1H, H-9), 7.02 (d, *J* = 8.2 Hz, 1H, H-8), 6.98 (dt, *J* = 7.3 Hz, 1H, H-10), 5.92 (s, 1H, H-4), 4.21 (t, *J* = 2.8 Hz, 1H, H-12), 2.28 (dd, *J* = 13.5 and 3.0 Hz, 1H, H-13A), 2.34 (dd, *J* = 13.5 and 3.0 Hz, 1H, H-13B), 2.16 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.2, 162.6, 161.1, 151.2, 138.3, 135.4, 128.7, 128.3, 128.1, 127.1, 125.4, 122.2, 116.2, 103.3, 99.7, 99.6, 32.8, 26.6, 19.9 ppm. HRMS (TOF): calcd. for $C_{21}H_{16}ClO_4$ $(M+H)^+$ 367.0737; found 367.0747. Anal. calcd for $C_{21}H_{15}ClO_4$: C, 68.77; H, 4.12. Found: C, 68.48; H, 4.09.

10-Chloro-3-methyl-6-phenyl-6,12-methanobenzo[*d*]-pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-one (7e): Colorless crystals (264 mg, 72 % yield). M.p. 123-124 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.65-7.62 (m, 2H, Ar-H), 7.47-7.45 (m, 4H, Ar-H), 7.14 (dd, *J* = 8.7 and 2.5 Hz, 1H, H-9), 6.96 (d, *J* = 8.7 Hz, 1H, H-8), 5.94 (s, 1H, H-4), 4.19 (t, *J* = 2.7 Hz, 1H, H-12), 2.39-2.27 (m, 2H, CH_2), 2.20 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.1, 162.6, 161.1, 151.2, 138.3, 135.4, 128.7, 128.3, 128.1, 127.1, 125.4, 122.2, 116.2, 103.3, 99.7, 99.6, 32.8, 26.6, 19.9 ppm. HRMS (TOF): calcd. for $C_{21}H_{16}ClO_4$ $(M+H)^+$ 367.0737; found 367.0747.

10-Chloro-3-methyl-6-(*p*-tolyl)-6,12-methano-benzo[*d*]-pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-one (**7f**)**:** Colorless crystals (304 mg, 80 % yield). M.p. 126-127 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.52 (d, *J* = 8.2 Hz, 2H, H-2′,6′), 7.46 (d, *J* = 2.4 Hz, 1H, H-11), 7.27 (d, *J* = 8.1 Hz, 2H, H-3′, 5′), 7.13 (dd, *J* = 8.7 and 2.5 Hz, 1H, H-9), 6.95 (d, *J* = 8.6 Hz, 1H, H-8), 5.92 (s, 1H, H-4), 4.17 (br. s, 1H, H-12), 2.41 (s, 3H, 4′-CH_3), 2.37-2.25 (m, CH_2 2H), 2.19 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.1, 163.0, 161.2, 150.1, 139.4, 136.4, 129.2, 128.7, 128.6, 128.1, 127.6, 127.1, 126.8, 125.4, 117.5, 102.7, 100.1, 99.8, 32.4, 26.6, 21.2, 19.9 ppm. HRMS (TOF): calcd. for $C_{22}H_{17}ClNaO_4$ $(M+Na)^+$ 403.0713; found 403.0679.

10-Chloro-6-(4-methoxyphenyl)-3-methyl-6,12-methano-benzo[d]pyrano[3,4-g][1,3]dioxocin-1(12H)-one (7g): Colorless crystals (329 mg, 83 % yield). M.p. 152-153 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.55 (d, *J* = 8.7 Hz, 2H, H-2′ and H-6′), 7.46 (d, *J* = 2.4 Hz, 1H, H-11), 7.12 (dd, *J* = 8.4 and 2.4 Hz, 1H, C-9), 6.97 (d, *J* = 8.7 Hz, H-3′ and H-5′), 6.94 (1H, d, *J* = 8.7 Hz, H-8), 5.91 (1H, s, 2H, H-4), 4.17 (br. s, 1H, H-12), 3.85 (s, 3H, OMe), 2.35-2.26 (m, 2H, CH_2), 2.91 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.1, 163.0, 161.2, 160.4, 150.1, 131.5, 128.1, 127.6, 127.1, 126.9, 126.8, 117.5, 113.8, 102.7, 100.1, 99.8, 55.4, 32.5, 26.7, 19.9 ppm. HRMS (TOF): calcd. for $C_{22}H_{17}ClNaO_5$ $(M+Na)^+$ 419.0662; found 419.0647.

3,10-Dimethyl-6-(*p*-tolyl)-6,12-methanobenzo[*d*]-pyrano [3,4-*g*][1,3]dioxocin-1(12*H*)-one (7h): Colorless crystals (284 mg, 79 % yield). M.p. 121-122 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.54 (d, *J* = 8.4 Hz, 2H,H-2′,6′),7.36-7.24 (m, 2H, Ar-H), 6.97 (dt, *J* = 8.1 and 1.8 Hz, 1H, H-9), 6.93 (d, 1H, *J* = 8.2 Hz, H-8), 5.91 (s, 1H, H-4), 4.17 (t, *J* = 2.7 Hz, 1H, H-12), 2.40 (s, 3H, 4′-CH_3), 2.35-2.23 (m, 2H, CH_2), 2.30 (s, 3H, 10-CH_3), 2.17 (3H, s, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.4, 163.0, 160.8, 149.2, 139.2, 137.0, 131.4, 129.1, 128.7, 128.3, 125.5, 125.3, 116.0, 103.3, 100.1, 100.0, 32.9, 26.7, 21.2, 20.5, 19.9 ppm. HRMS

(ESI): calcd. for $C_{23}H_{20}O_4$ $(M+Na)^+$ 383.1259; found 383.0748; Anal. calcd for $C_{23}H_{20}O_4$: C, 76.65; H, 5.59. Found: C, 76.70; H, 5.54.

6-(4-Methoxyphenyl)-3,10-dimethyl-6,12-methanobenzo-[*d*]pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-one (7i): Colorless crystals (305 mg, 81 % yield) M.p. 135-136 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 8.4 Hz, 2H, H-2′,6′), 7.28 (s, 1H, H-11), 6.97-6.92 (m, 3H, Ar-H), 6.91 (d, *J* = 8.1 Hz, 1H, H-8), 5.90 (s, 1H, H-4), 4.16 (br. s, 1H, H-12), 3.84 (s, 3H, 4′-OCH_3), 2.28 (s, 3H, 10-CH_3), 2.34-2.22 (m, 2H, CH_2), 2.17 (s, 3H, 3-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.4, 163.0, 160.3, 149.2, 132.1, 131.4, 128.7, 128.3, 126.9, 125.3, 116.0, 113.8, 103.3, 100.1, 99.9, 55.4, 33.0, 26.8, 20.5, 19.9. HRMS (ESI): calcd. for $C_{23}H_{21}O_5$ $(M+Na)^+$ 399.1208; found 399.1067; Anal. calcd for $C_{23}H_{20}O_5$: C, 73.39; H, 5.36. Found: C, 73.42; H, 5.08.

8-Methoxy-3-methyl-6-phenyl-6,12-methanobenzo[*d*] pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-one (7j): Colorless crystals (217 mg, 60 % yield). M.p. 142-143 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.72-7.68 (m, 2H, Ar-H), 7.48-7.43 (m, 4H, Ar-H), 6.93 (dt, *J* = 7.5 and 1.5 Hz, 1H, H-11), 6.81 (d, *J* = 8.1 Hz, 1H, H-9), 5.91 (s, 1H, H-4), 4.23 (t, *J* = 3 Hz, 1H, H-12), 3.87 (s, 3H, 8-OCH_3), 2.38-2.23 (m, 2H, CH_2), 2.17 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.3, 163.0, 160.9, 148.1, 140.7, 139.7, 129.2, 128.5, 126.7, 125.8, 121.8, 119.9, 110.9, 103.1, 100.0, 99.9, 56.1, 32.9, 26.7, 19.9 ppm. HRMS (ESI): calcd. for $C_{22}H_{19}O_5$ $(M+H)^+$ 363.1232; found 363.1419.

8-Methoxy-3-methyl-6-(*p*-tolyl)-6,12-methanobenzo[*d*]-pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-one (7k): Colorless crystals (241 mg, 64 % yield). M.p. 145-146 °C. IR (KBr): ν = 1713, 1649, 1585, 1487, 1443, 1399, 1338, 1271, 1251, 1207, 1184, 1140, 1116, 1018, 995, 944, 863, 816, 769 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.57 (d, *J* = 8.1 Hz, 2H, H-2′,6′), 7.25 (d, *J* = 7.8 Hz, 2H, H-3′,5′), 7.09 (d, *J* = 7.5 Hz, 1H, H-11), 6.91 (t, *J* = 7.8 Hz, 1H, H-10), 6.79 (d, *J* = 8.1 Hz, 1H, H-9), 5.90 (s, 1H, H-4), 4.21 (br. t, *J* =2.4 Hz, 1H, H-12), 3.85 (s, 3H, 8-OCH_3), 2.39 (s, 3H, 4′-CH_3), 2.36-2.24 (m, 2H, CH_2), 2.16 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.3, 163.1, 160.8, 148.1, 140.7, 139.1, 136.8, 129.1, 126.7, 125.6, 121.7, 119.9, 110.9, 103.1, 100.0, 99.9, 56.1, 32.9, 26.8, 21.2, 19.9 ppm. HRMS (ESI): calcd. for $C_{23}H_{21}O_5$ $(M+H)^+$ 377.1389; found 377.1641.

3-Methyl-6-(thiophen-2-yl)-6,12-methanobenzo-[*d*]pyrano-[3,4-*g*][1,3]dioxocin-1(12*H*)-one (7l): Colorless crystals (302 mg, 78 % yield). M.p. 123-124 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.47 (br. d, *J* = 6.7 Hz, 1H, H-11), 7.40 (d, *J* = 4.3 Hz, 1H, H-5′), 7.30 (br. d, *J* = 2.7 Hz, 1H, H-2′), 7.18 (t, *J* = 6.9 Hz, 1H, H-9), 7.07 (br. t, *J* = 3.9 Hz, 1H, H-4′), 7.00 (d, *J* = 7. 1H, 6 Hz, H-8), 6.95 (t, *J* = 7.4 Hz, 1H,H-10), 5.90 (s, 1H, H-4), 4.24 (br. s, 1H, H-12), 2.52 (dd, *J* = 13.5 and 2.7 Hz, 1H, H-13A), 2.45 (dd, *J* = 13.5 and 3 Hz, 1H, H-13B), 2.17 (s, 3H, 3-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.2, 162.5, 161.0, 151.0, 142.9, 128.2, 128.0, 127.0, 126.5, 125.4, 125.3, 122.2, 116.3, 103.3, 99.8, 98.9, 33.0, 26.7, 19.9 ppm. Anal. calcd for $C_{19}H_{14}O_4S$: C, 67.44; H, 4.17. Found: C, 67.27; H, 4.26.

3,6-Dimethyl-6,12-methanobenzo[*d*]pyrano[3,4-*g*][1,3]-dioxocin-1(12*H*)-one (8a): Colorless crystals (197 mg, 73 % yield). M.p. 112-113 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.41 (br. d, *J* = 7.4 Hz, 1H, H-11), 7.12 (br. t, *J* = 7.8 Hz, 1H, H-9), 6.90 (d, *J* = 7.4 Hz, 1H, H-8), 6.85 (t, *J* = 8.1 Hz, 1H, H-10), 5.79 (s, 1H, H-4), 4.12 (br. s, 1H, H-12), 2.19 (dd, *J* = 13.5 and 2.7 Hz, 1H, H-13A), 2.14 (s, 3H, 3-CH_3), 2.10 (dd, *J* = 13.5 and 2.7 Hz, 1H, H-13B), 1.85 (s, 3H, 6-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.3, 162.7, 160.7, 151.1, 128.0, 125.4, 121.6, 116.0, 103.2, 99.8, 99.4, 30.8, 26.7, 26.2, 19.8 ppm. HRMS (ESI): calcd. for $C_{16}H_{15}O_4$ $(M+Na)^+$ 293.0790; found 293.0454. Anal. calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22%; Found: C, 71.27; H, 5.37.

6-Ethyl-3-methyl-6,12-methanobenzo[*d*]pyrano[3,4-*g*]-[1,3]dioxocin-1(12*H*)-one (8b): Colorless crystals (168 mg, 77 % yield). M.p. 115-116 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.41 (br. d, *J* = 7.1 Hz, 1H, H-11), 7.11 (br. t, 1H, *J* = 6.0 Hz, H-9), 6.92-6.82 (m, 2H, H-8 and H-10), 5.80 (s, 1H, H-4), 4.12 (br. s, 1H, H-12), 2.20-2.12 (m, 2H, CH_2), 2.14 (s, 3H, 3-CH_3), 1.43 (br. s, 1H, H_B of 6-CH_2-), 0.91 (br. s, 1H, H_A of 6-CH_2-), 0.66 (br. s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.4, 162.8, 160.7, 151.2, 127.9, 125.9, 121.6, 115.9, 103.3, 99.8, 99.7, 30.7, 26.3, 19.8, 18.5, 1.2, 0.8 ppm. HRMS (TOF): calcd. for $C_{17}H_{17}O_4$ $(M+H)^+$ 285.1127; found 285.1107.

6-Isobutyl-3-methyl-6,12-methanobenzo[*d*]pyrano-[3,4-*g*]-[1,3]dioxocin-1(12*H*)-one (8c): Colorless crystals (234 mg, 75 % yield). M.p. 114-115°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36 (br. d, *J* = 7.3 Hz, 1H, H-11), 7.05 (br. t, *J* = 7.8 Hz, 1H, H-9), 6.85-6.79 (m, 2H, H-8 and H-10), 5.69 (s, 1H,H-4), 4.05 (t, 1H, *J* = 2.5 Hz, H-12), 2.05 (s, 3H, 3-CH_3), 2.14-1.98 (m,3H), 1.98 (br. s, 2H, 6-CH_2-), 1.02 (br. s, 6H, 2×CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.3, 162.6, 160.7, 151.1, 127.94, 127.9, 125.7, 121.5, 116.0, 103.1, 101.3, 99.8, 47.6, 29.4, 26.0, 24.1, 23.9, 23.8, 19.8 ppm. Anal. calcd for $C_{19}H_{20}O_4$: C, 73.06; H, 6.45. Found: C, 73.11; H, 6.39.

6-Cyclopropyl-3-methyl-6,12-methanobenzo[*d*]pyrano-[3,4*g*][1,3]dioxocin-1(12*H*)-one (8d): Colorless crystals (213 mg, 72 % yield). M.p. 110-111°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.41 (1H, d, *J* = 6.6 Hz, Ar-H), 7.12 (1H, t, *J* = 7.0 Hz, Ar-H), 6.89 (2H, m, Ar-H), 5.80 (1H, s, H-4), 4.13 (1H, br. s, Aliph. CH), 2.14 (3H, s, 3-CH_3), 2.15-2.02 (3H,

m, CH of cyclopropyl unit and $-CH_2-$ of chroman unit), 1.11 (4H, t, J = 7.3Hz, $-CH_2-CH_2-$ of cyclopropyl unit) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.4, 162.9, 160.6, 151.4, 128.0, 125.7, 121.5, 116.0, 103.3, 101.3, 99.9, 32.5, 28.5, 25.9, 19.8, 7.6 ppm. HRMS (TOF): calcd. for $C_{18}H_{17}O_4$ $(M+H)^+$ 297.1127; found 297.1110.

Recovery of the Catalyst

The catalyst amberlyst-15 separated from the reaction mixture was washed successively with ethyl acetate and acetone till the washings became colorless. It was then dried in a hot air oven at 100°C for 10 h. The dried catalyst was used in the next cycle.

UV-vis Absorption Spectroscopy

UV-Visible study of DNA/compound **7b** and **7c** system was performed on a SIMADZU UV1700 at room temperature (25^0C) with a four-clear-sided quartz cell (2 mL) of 1 cm path length. The respective spectra were recorded with a matched pair of silica cuvettes over the wavelength range 200-600 nm against a solvent blank reference.

Fluorescence Spectroscopy

Fluorescence titrations were carried out at constant concentration of **7b** and **7c** (10 μM) and with the gradual addition of DNA (1 to 20 μM) at pH 7.4 using Shimadzu RF-5301 PC at room temperature. Emission spectra were recorded from 315 to 550 nm with an excitation wavelength of 295 nm.

Emission spectra for iodide quenching experiments were recorded in presence and/or absence of 20 μM DNA. In this experiment, fluorescence spectra from 300–500 nm of 50 μM **7b** and **7c** in 10 μM phosphate buffer (pH 7.4) were taken with varying concentration of KI between 0-8 mM at 280 nm excitation wavelength.

In case of EB displacement assay, a solution of EB-DNA complex which was formed by mixing 2 μM of EB and 20 μM of ct-DNA, was titrated with increasing concentration of **7b** and **7c** (excited at 471 nm and emission recorded from 550–850 nm).

The fluorescence studies for the compounds **7 / 8** with 10 μM β-lg was done at room temperature. Here, the spectral changes of β-lg were noticed with successive addition of **7 / 8** to β-lg. The excitation wavelength of β-lg (10 μM, with 10 mM phosphate buffer, pH 7.4, with 5% ethanol) was 295 nm and emission scans were recorded in the range 310–400 nm.

Docking Study

The molecular docking studies of **7b** and **7c** into the three dimensional structures of ct-DNA and of **7c** and **7k** with β-lg were carried out using Auto Dock 4.2.0 software package. The energy minimized conformation of each of **7b**, **7c** and **7k**, used for docking study, was obtained from DFT optimization. The docking study was done using an efficient and durable algorithm, Lamarckian Genetic Algorithm (LGA) for 126 x 126 x 126 grid box.

Study of Antibacterial Properties

Antimicrobial activities of the compounds **7b**, **7c**, **7k** and **8a** were tested using the well diffusion method. The liquid Nutrient Agar (NA) medium was poured on Petri dishes and it was allowed to solidify under laminar airflow chamber. A sterile swab was used to evenly distribute 1mL microbial culture over the agar surface. The plates were subjected to drying for 15 minutes before two wells of 0.5 cm diameter were made in the agar medium using a sterile cork borer. A solution of each of the said compounds (50 μl) was placed into each well. All the plates were then placed at 37°C incubator for 24 h. The inhibition zones were measured with a caliper.

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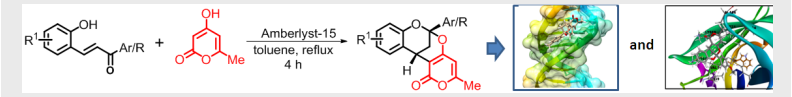

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Synthesis of 6,12-Methanobenzo-[*d*]pyrano[3,4-*g*][1,3]dioxocin-1(12*H*)-ones and Study of Their Interaction with DNA and β-Lactoglobiulin

Sixteen 6,12-methanobenzo[*d*]pyrano-[3,4-*g*][1,3]dioxocin-1(12*H*)-ones, a new class of 2,8-dioxabicyclo[3.3.1]nonanes, have been synthesized and fully characterized. Among them two show significant ct-DNA-binding property and all show strong binding with β-lactoglobulin. Study done on four compounds, shows that two of them exhibit moderate antibacterial properties.

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