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ORIGINAL ARTICLE

Naphthyridine-based receptors for flurometric detection of urea and biotin

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Abstract Naphthyridine-based receptors **1**–**4** have been designed and synthesized for the recognition of urea in $CHCl_3$ containing 1% CH_3CN. Receptor **1** also binds biotin and its methyl ester with moderate binding constant values. In comparison, receptor **2** is less efficient in recognising biotin and its methyl ester analogue. Receptor **1** binds urea and biotin with binding constant values of 1.02×10^4 and 1.08×10^4 M^{-1}, respectively, in $CHCl_3$ containing 1% CH_3CN and shows significant change in emission of naphthyridine upon complexation. Characterization and sensing properties of the receptors were evaluated by ^1H NMR, UV–vis and fluorescence spectroscopic methods.

Keywords Biotin recognition · Urea recognition · Fluorometric detection · Naphthyridine-based receptors

Introduction

Recognition of individual molecules is one of the most fundamental processes both in chemistry and biology. The study of synthetic receptors that mimic the biological processes is important in the area of molecular recognition [1, 2]. In designing such receptors judicious arrangement of hydrogen-bonding sites has received a great deal of recent attention. Considerable efforts have been made in last few decades to design receptors for bioactive substrates like urea [3], biotin [4], carbohydrates [5], carboxylic acids [6], amino acids [7] etc. Among these substrates of biological significance, urea is important one because it is toxic, pollutant and causes serious biological disorders [8, 9]. It has a well-defined geometry, and it can form at least six hydrogen bonds. The detection of this small molecule by suitable synthetic receptor is therefore important. In this regard, very few fluorescent receptors for urea recognition are known in the literature. Goswami et al. reported a pyridine-based macrocyclic receptor for the fluorometric detection of urea [10]. Chetia and Iyer showed that a 2,6-bis(2-benzimidazole)pyridine receptor is able to bind urea and exhibits a concomitant change in fluorescence [11]. Our anthracene-based open and macrocyclic receptors for fluorometric detection of urea are mentionable [12]. Non-fluorescent receptors such as crown ether-based receptors, developed by Pedersen [3] and carboxylic acid containing crown ether receptor of Reinhoudt [13] are known to bind urea. Reinhoudt et al. introduced the concept of using an electrophilic center to bind urea in the cavity of crown ether [14]. Bell et al. synthesized polyaza heterocycles which were effective for urea recognition [15]. Recently, we have shown that a pyridine amide-based macrocyclic receptor can form a strong inclusion complex with urea [16]. Goswami et al. reported the solubilization and recognition of urea using a simple dinaphthyridine receptor [17].

Similarly, among the substrates biotin (referred to as vitamin H in human) is an essential cofactor for a number of enzymes that have diverse metabolic functions [18]. Structurally it consists of a pentanoic acid side chain and a *cis*-fused bicyclic moiety with sulfur atom in the ring, as evidenced from crystallographic studies [19]. The crystal structure of biotin shows that the carboxyl group of one biotin molecule is intermolecularly hydrogen bonded to the

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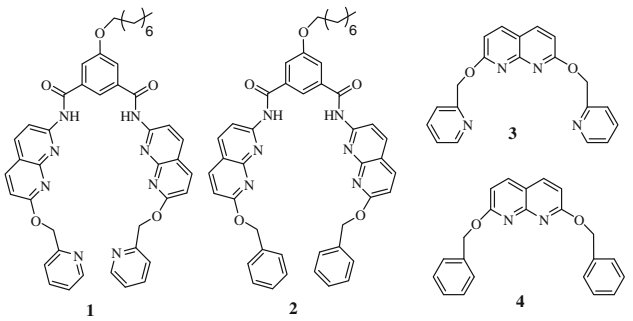
urea linkage of the other biotin molecule. The valeryl chain is severely twisted from the maximally extended all *trans*-conformation. In an effort to bind this interesting biological substrate, careful design of molecular receptor that can bind both the urea and acid parts together is necessary. A limited number of designed receptors are known in the literature. In this aspect, the use of Troger's base receptor as reported by Wilcox and co-workers was noteworthy [20]. Claramunt et al. reported the recognition of biotin methyl ester [21]. Goswami et al. demonstrated that pyridine amide-based simple receptor is able to bind biotin itself involving both carboxylic acid and cyclic urea part of biotin [4]. We have also recently reported the recognition of biotin by a number of synthetic receptors, which are benzthiazole [22] and naphthyridine [23] based.

Thus the noteworthy progress made in this horizon inspired us to work on the recognition of urea and biotin. In this paper, we report the design and synthesis of a series of naphthyridine-based receptors for recognition of urea, biotin and their derivatives.



1 2 3 4

Materials and methods

Materials

All the solvents were dried by usual procedures prior to use. All the reactions were carried out under nitrogen. IR and UV spectra were recorded on Perkin Elmer model L120-00A and Lambda-25, respectively. Fluorescence was recorded by PerkinElmer LS55 instrument. For ^{1}H and ^{13}C NMR spectra Bruker 400 and 500 MHzs were used. Melting points were recorded in open capillaries and are uncorrected.

Synthesis of **1–4**

*7-(Pyridin-2-ylmethoxy)-1,8-naphthyridin-2-amine (**6a**)*

To a stirred solution of 2-hydroxymethylpyridine (0.6 g, 0.005 mol) in dry THF, NaH (0.14 g, 0.006 mol) was added and refluxed for 3 h under nitrogen atmosphere. After cooling, 2-acetamido-7-chloro-1, 8-naphthyridine **5a** (1.45 g, 0.006 mol) was added followed by catalytic amount of Cu_2O. Reflux was continued for further 18 h. The desired amine **6a** was isolated in 60% yield after extraction with chloroform followed by purification through column chromatography using 35% ethyl acetate in petroleum ether (60–80 °C). Mp was noted as 170 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, 1H, $J = 4.36$ Hz), 7.80 (m, 2H), 7.69 (t, 1H), 7.48 (d, 1H, $J = 7.8$ Hz), 7.22 (m, 1H), 6.82 (d, 1H, $J = 8.56$ Hz), 6.61 (d, 1H, $J = 8.48$ Hz), 5.69 (s, 2H), 4.96 (s, 2H). FTIR (ν cm^{-1}, KBr): 3473, 3364, 3188, 2913, 1638, 1595, 1512, 1432, 1324, 1246, 1137.

*7-(Benzyloxy)-1,8-naphthyridin-2-amine (**6b**)*

A solution of benzyl alcohol (0.3 g, 0.003 mol) in dry THF was stirred with NaH (0.07 g, 0.003 mol) for 2 h under mild reflux. To it *N*-(7-chloro-1,8-naphthyridin-2-yl) butyramide **5b** (0.76 g, 0.003 mol), dissolved in dry THF was added followed by catalytic amount of Cu_2O at RT. Reaction was continued for further 16 h at refluxing condition. Solvent was removed under reduced pressure; residue was dissolved in $CHCl_3$ (35 mL) and washed with water. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and the crude mass was purified through column chromatography [eluent: 50% ethyl acetate in petroleum ether (60–80 °C)]. The desired product was obtained as semi solid (0.47 g, 62% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, 1H, $J = 8.5$ Hz), 7.75 (d, 1H, $J = 8.7$ Hz), 7.49 (d, 2H, $J = 7.4$ Hz), 7.38 (t, 2H, $J = 7.6$ Hz), 7.32 (t, 1H, $J = 7.4$ Hz), 6.75 (d, 1H, $J = 8.5$ Hz), 6.59 (d, 1H, $J = 8.5$ Hz), 5.58 (s, 2H), 5.08 (s, 2H). FTIR (ν cm^{-1}, KBr): 3336, 2926, 1671, 1627, 1601, 1513, 1382, 1323, 1270, 1139.

*5-octyloxy-N1,N3-bis(7-(pyridin-2-ylmethoxy)-1,8-naphthyridin-2-yl)isophthalamide (**1**)*

In dry THF solution of 5-octyloxyisophthaloyldichloride (0.2 g, 0.001 mol), a solution of 7-(pyridin-2-ylmethoxy)-1,8-naphthyridin-2-amine **6a** (0.46 g, 0.002 mol) and Et_3N (0.26 mL, 0.002 mol) in dry THF was added dropwise with constant stirring. The reaction was continued for 18 h. Solvent was removed under reduced pressure, washed with 20% $NaHCO_3$ solution and extracted with $CHCl_3$ containing 3% CH_3OH. The organic layers were collected, washed with water and dried over anhydrous Na_2SO_4. After evaporation of solvent it was passed through a silica gel column (eluent: 3% CH_3OH in $CHCl_3$) and receptor **1** was obtained as off white solid (0.09 g, 20% yield). Mp 124 °C.

^{1}H NMR (500 MHz, $CDCl_3$) δ : 9.17 (2H, s, –NHCO–), 8.64 (2H, d, $J = 5$ Hz), 8.58 (2H, d, $J = 10$ Hz), 8.19 (3H, d, $J = 10$ Hz), 8.04 (2H, d, $J = 10$ Hz), 7.72 (2H, t,

J = 10 Hz), 7.68 (2H, s), 7.52 (2H, d, J = 10 Hz), 7.25–7.23 (2H, m), 7.07 (2H, d, J = 10 Hz), 5.74 (4H, s), 4.11 (2H, t, J = 5 Hz), 1.89–1.82 (m, 2H), 1.53–1.50 (m, 2H), 1.40–1.30 (m, 8H), 0.90 (t, 3H, J = 5 Hz). ^{13}C (125 MHz, $CDCl_3$) 165.1, 160.4, 156.8, 154.4, 149.8, 139.5, 139.3, 137.1, 136.2, 123.2, 122.6, 118.7, 118.2, 117.8, 117.6, 113.4, 113.3, 112.9, 69.3, 69.0, 32.2, 29.7, 29.6, 29.5, 26.3, 23.1, 14.5. FTIR (ν cm^{-1}, KBr): 3363, 3062, 2922, 2851, 1682, 1612, 1458, 1187, 1133. Mass (ESI): 763.2 $(M + H)^+$, 556.3, 425.2, 382.3, 362.2.

*N1,N3-Bis(7-(benzyloxy)-1,8-naphthyridin-2-yl)-5-propoxyisophthalamide (**2**)*

A solution of 7-(benzyloxy)-1,8-naphthyridin-2-amine **6b** (0.45 g, 0.002 mol) was added to a dry THF solution of 5-octyloxyisophthaloyldichloride (0.2 g, 0.001 mol) along with Et_3N (0.26 mL, 0.002 mol). The reaction mixture was stirred at RT for 14 h. Solvent was evaporated under vacuum, neutralized with saturated $NaHCO_3$ solution and extracted with $CHCl_3$. Organic layers were separated, washed with water and dried over anhydrous Na_2SO_4. The layer was concentrated and purified through column chromatography using 2% CH_3OH in $CHCl_3$. Receptor **2** was obtained as yellow solid (0.41 g, 40% yield). Mp 104 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ : 9.06 (s, 2H), 8.58 (d, 2H, J = 8.5 Hz), 8.19 (d, 2H, J = 8.8 Hz), 8.17 (s, 1H), 8.02 (d, 2H, J = 8.6 Hz), 7.67 (s, 2H), 7.51 (d, 4H, J = 7.2 Hz), 7.42–7.33 (m, 6H), 6.99 (d, 2H, J = 8.6 Hz), 5.61 (s, 4H), 4.11 (t, 2H, J = 6.4 Hz), 1.88–1.85 (m, 2H), 1.37-1.19 (m, 10H), 0.88 (t, 3H, J = 6.3 Hz). ^{13}C (100 MHz, $CDCl_3$) 165.1, 164.6, 160.0, 154.1, 152.9, 139.2, 138.7, 136.5, 135.9, 128.5, 128.2, 128.1, 118.2, 117.1, 117.06, 113.04, 112.2, 68.9, 68.5, 31.8, 29.3, 29.2, 29.01, 25.9, 22.6, 14.1. FTIR (ν cm^{-1}, KBr): 3364, 3063, 2923, 2853, 1682, 1614, 1531, 1505, 1439, 1326, 1274, 1131. Mass (ESI): 760.9 $(M + H)^+$, 597.4, 583.6, 542.5.

*2,7-Bis(pyridin-2-ylmethoxy)-1, 8-naphthyridine (**3**)*

A dry THF solution of 2-hydroxymethylpyridine (166 mg, 1.51 mmol) was stirred with NaH (45 mg, 1.87 mmol) under mild refluxing condition for 1.5 h. To this solution 2,7-dichloro-1,8-naphthyridine (100 mg, 0.5 mmol) was added along with catalytic amount of Cu_2O. The reaction was further continued for 10 h. Solvent was removed under vacuum and the solid mass was redissolved in $CHCl_3$ (30 mL). The organic layer was collected, washed with water (3 × 20 mL), dried over anhydrous Na_2SO_4 and finally concentrated under reduced pressure. The residue was purified by column chromatography (eluent: 40% ethyl acetate in petroleum ether (60 – 80 °C)) to give compound **3** as brown solid (95 mg, 55% yield). Mp 72 °C.

^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.66 (d, 2H, J = 4.8 Hz), 7.96 (d, 2H, J = 8.6 Hz), 7.72 (t, 2H, J = 7.7 Hz), 7.54 (d, 2H, J = 7.8 Hz), 7.24 (t, 2H, J = 6.7 Hz), 6.98 (d, 2H, J = 8.6 Hz), 5.75 (s, 4H).^{13}C (125 MHz, $CDCl_3$) δ: 164.9, 157.2, 154.7, 149.8, 139.4, 137.1, 123.1, 122.7, 115.9, 111.5, 69.2. FT-IR ν cm^{-1} (KBr): 3417, 2925, 2850, 1611, 1572, 1502, 1433, 1327. UV (1% CH_3CN in $CHCl_3$, c = 2.44 × 10^{-5} M) λ_{max} (nm) 286, 292, 298, 304, 311, 318, 326, 344. Mass (ESI): 345.2 $(M + H)^+$, 367.2 $(M + Na)^+$.

*2,7-Bis(benzyloxy)-1, 8-naphthyridine (**4**)*

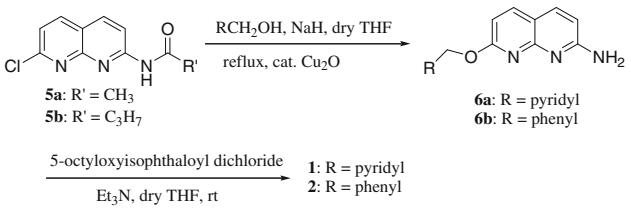
In dry THF, benzyl alcohol (136 mg, 1.26 mmol) was stirred with NaH (36 mg, 1.5 mmol) for 1 h under mild refluxing condition. A solution of 2,7-dichloro-1,8-naphthyridine (100 mg, 0.5 mmol) in dry THF was added to it at RT and reflux was continued for further 8 h. The solvent was evaporated, washed with water and extracted with $CHCl_3$ (3 × 20 mL). The organic layers were taken together, left over anhydrous Na_2SO_4 and concentrated. After purification of the crude mass through column chromatography using 20% ethyl acetate in petroleum ether (60–80 °C) as eluent, compound **4** was isolated as white solid (110 mg, 64% yield). Mp 100 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (d, 2H, J = 8.6 Hz), 7.51 (d, 4H, J = 7.2 Hz), 7.41 (t, 4H, J = 8.0 Hz), 7.33 (t, 2H, J = 8.0 Hz), 6.91 (d, 2H, J = 8.6 Hz), 5.62 (s, 4H).^{13}C (100 MHz, $CDCl_3$) δ 164.9, 154.6, 138.8, 136.9, 128.5, 128.2, 127.9, 115.3, 111.1, 68.4. FT-IR ν cm^{-1} (KBr): 3418, 3063, 3031, 2925, 1607, 1560, 1502, 1435, 1326, 1259. UV (1% CH_3CN in $CHCl_3$, c = 2.44 × 10^{-5} M) λ_{max} (nm) 293, 299, 305, 312, 319, 326. Mass (ESI): 343.4 $(M + H)^+$, 365.4 $(M + Na)^+$.

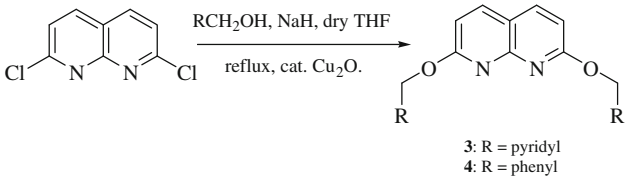
Results and discussion

Synthesis

Receptors **1** [24] and **2** were synthesized according to the Scheme 1. Coupling of 2-acetylamino-7-chloro-1,8-naphthyridine **5a**, obtained by known method [25], with 2-hydroxymethylpyridine in dry THF in the presence of NaH and a catalytic amount of Cu_2O gave compound **6a**. Reaction of amine **6a** with 5-octyloxyisophthaloyl diacid chloride gave the receptor **1** in 20% yield. The same reaction conditions were applied to the synthesis of receptor **2** (Scheme 1). Here, 2-butrylamino-7-chloro-1,8-naphthyridine **5b** was coupled with benzyl alcohol, under the same reaction condition to give amine **6b** as the sole product. The synthesis of compounds **3** and **4** was started with 2,7-dichloro-1,8-naphthyridine which was obtained by known method [25]. Coupling of 2-hydroxymethylpyridine



Scheme 1 The syntheses of receptors **1** and **2**



Scheme 2 The syntheses of receptors **3** and **4**

and benzyl alcohol with 2,7-dichloro-1,8-naphthyridine in dry THF in the presence of NaH and catalytic amount of Cu_2O afforded the receptors **3** and **4** in 55% and 64% yield, respectively (Scheme 2).

Molecular modeling

In the designed receptors naphthyridine has been taken as hydrogen bonding site as well as fluorescent probe for monitoring the recognition event.

The optimization of the geometries of **1** and **2** was performed by MMX calculation.[1] It is evident from Fig. 1 that the open cleft of **1** is able to make a strong complex with both urea and biotin utilizing hydrogen bond donors and acceptors together. Similarly, receptor **2**, which does not have pyridine rings at the lower rim, also interacts with urea in the cleft involving same number of hydrogen bonds like receptor **1**. Only the difference in between the complexes of urea with receptors **1** and **2** is lying with the marginal change in hydrogen bond distances (see Fig. 1) which influence the receptor **1** to from a stable urea complex. Due to absence of hydrogen bonding site at the lower rim of **2**, biotin is complexed at the diamide core involving only the cyclic urea part leaving carboxylic acid uncomplexed (Fig. 1d). The energy values of the complexes of **1** and **2** and the intermolecular hydrogen bond distances are displayed in Fig. 1. MMX calculations of the complexes of **1** and **2** with *N*,*N*′-dimethylurea and thiourea were also carried out in gas phase (see Supplementary Material) and they were found to be less stable due to formation of less number of hydrogen bonds.

[1] Energy minimization was carried out using MMX (PC Model Serena Software (1993). Molecular modeling was performed using standard constants, and the dielectric constant was maintained at 1.5.

Complexation properties

^{1}H-NMR study

However, to understand the binding properties of the receptors in solution, ^{1}H NMR spectra were recorded in dry $CDCl_3$. In each case, powder urea was added to $CDCl_3$ solutions of the receptors, followed by thorough sonication for about 10 min. The excess urea was removed by filtration, and the filtrate of each receptor solution was used to record the ^{1}H NMR spectra. In case of receptor **1** significant downfield shifts of the amide protons ($\Delta\delta = 2.05$) and isophthaloyl peri proton ($\Delta\delta = 0.52$) were observed in the presence of urea. In addition, a new peak at δ 6.37 for urea-protons was observed (Fig. 2). The proton integration ratio indicated the formation of 1:1 complex and importantly, dilution of this 1:1 complex in $CDCl_3$ did not show any change in ^{1}H NMR spectra thereby suggesting a strong complexation. In comparison to **1**, receptor **2** showed weak interaction as evidenced from the appearance of a weak signal at δ 5.55 for urea (Fig. 3) followed by small downfield shifts of amide protons and the isophthaloyl peri proton (H_b) ($\Delta\delta$ for amide NH 1.36 and $\Delta\delta$ for isophthaloyl peri proton 0.34; Fig. 3). The weak interaction is attributed due to the presence of phenyl ring, which does not have any hydrogen bond acceptor for a significant contribution to the interaction process. From the integration values it was found that receptor **2** was bound with $\sim$63% urea in $CDCl_3$. Thiourea, *N*, *N*′-dimethyl urea etc., were also individually added to the $CDCl_3$ solutions of **1** and **2**. After sonication and filtration, clear solutions were used to record the ^{1}H NMR spectra. Careful analysis of the results showed that thiourea, *N*, *N*′-dimethylurea interacted weakly with both receptors **1** and **2**. In ^{1}H NMR, weak signals for urea protons appeared at δ 4.55 and 4.45 during interactions with **3** and **4**, respectively. This suggested that receptors **3** and **4** are poor binder of urea.

A similar study was performed with biotin and its methyl ester. Figures 4 and 5 highlight the changes occurring during hydrogen bonding interactions of receptors **1** and **2** with biotin and biotin methyl ester. It is evident from Figs. 4 and 5 that the amide protons of both **1** and **2** are shifted more downfield in the presence of biotin methyl ester. In presence of biotin acid, the amide protons were shifted to the lesser extent. This is due to the existence of two possible modes of complexation of biotin with the receptors **1** and **2** in solution (Fig. 6). Receptor **1** may complex biotin *via* the modes **A** and **B**, which may remain in equilibrium in solution. Similarly, for receptor **2**, forms **C** and **D** are possible. In case of biotin methyl ester the possibility of hydrogen bonding of the ester group into the diamide core is negligible due to the bulky nature of the ester group. Therefore, while biotin has the chance to be

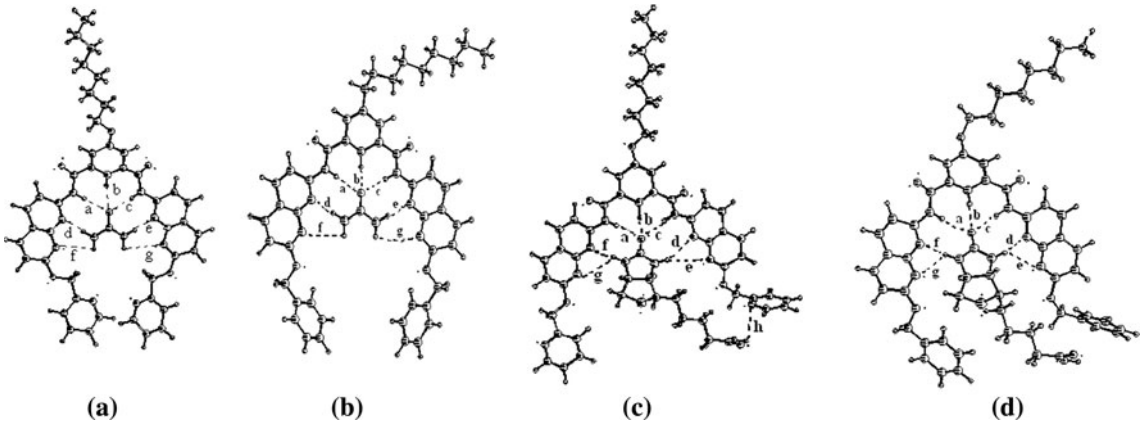


Fig. 1 Energy optimized geometries of (**a**) **1**. urea complex [E = 53.01 kcal/mol, a = 2.17 Å, b = 2.50 Å, c = 2.02 Å, d = 1.84 Å, e = 1.80 Å, f = 2.92 Å, g = 3.09 Å]; (**b**) **2**. urea [E = 56.08 kcal/mol, a = 2.18 Å, b = 2.45 Å, c = 2.02 Å, d = 1.82 Å, e = 1.81 Å, f = 3.07 Å, g = 3.2 Å]; (**c**). **1**. biotin [E = 80.81 kcal/mol, a = 2.21 Å, b = 2.41 Å, c = 2.28 Å, d = 2.69 Å, e = 3.36 Å, f = 1.85 Å, g = 2.94 Å, h = 2.99 Å]; (**d**) **2**. biotin [E = 68.50 kcal/mol, a = 2.31 Å, b = 2.57 Å, c = 1.96 Å, d = 1.89 Å, e = 2.72 Å, f = 1.88 Å, g = 2.55 Å]

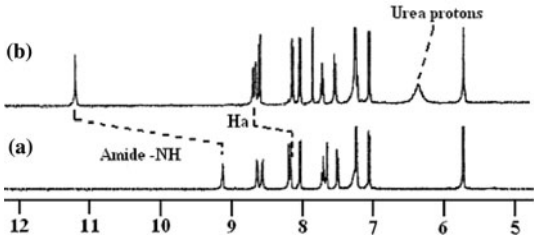


Fig. 2 (**a**) Partial ^{1}H NMR (400 MHz, $CDCl_3$) spectra of receptor **1** ($c = 2.81 \times 10^{-3}$ M). (**b**) 1:1 complex of receptor **1** with urea

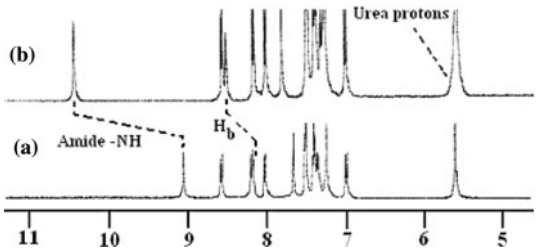


Fig. 3 (**a**) Partial ^{1}H NMR (400 MHz, $CDCl_3$) spectra of receptor **2** ($c = 2.82 \times 10^{-3}$ M). (**b**) 1:1 complex of receptor **2** with urea

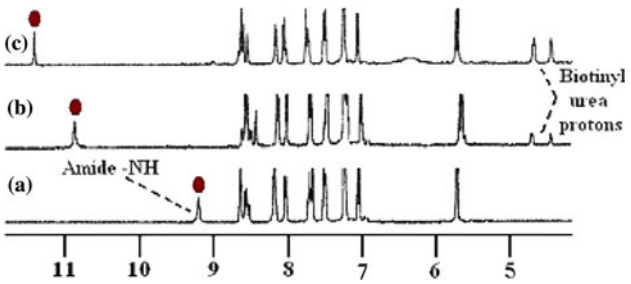


Fig. 4 (**a**) Partial ^{1}H NMR (400 MHz) spectra of receptor **1** ($c = 2.89 \times 10^{-3}$ M) and its 1:1 complex with (**b**) biotin and (**c**) biotin methyl ester in $CDCl_3$

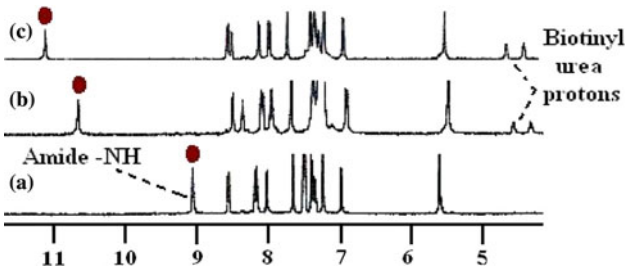


Fig. 5 (**a**) Partial ^{1}H NMR (400 MHz) spectra of receptor **2** ($c = 2.82 \times 10^{-3}$ M) and its 1:1 complex with (**b**) biotin acid and (**c**) biotin methyl ester in $CDCl_3$

complexed into the cleft of receptor **1** *via* the modes **A** and **B**, biotin methyl ester will follow only one binding mode (**A′**) giving the preference of complexation of cyclic urea part into the diamide core of **1** (Fig. 7). This is also true for receptor **2**. The pendant pyridyl groups in **1** play key role in the binding event. It is obvious from Fig. 6 that the dimaide core and the pendant pyridine rings are simultaneously involved in the binding of both acid and urea parts of biotin. In contrast, receptor **2** binds only one part (either acid or cyclic urea) of biotin and thus exhibits weak binding. However, amongst the different modes, modes **B** and **D** are only favourable due to compact binding of the cyclic urea motif with more number of hydrogen bonds.

After having this information from ^{1}H NMR, we carried out fluorescence and UV–vis titrations to understand the binding potencies, selectivities and sensitivities of the receptors with the guests mentioned in the study. All the guests were dissolved in $CHCl_3$ containing 1% CH_3CN and direct titration method was followed to evaluate the binding constant values.

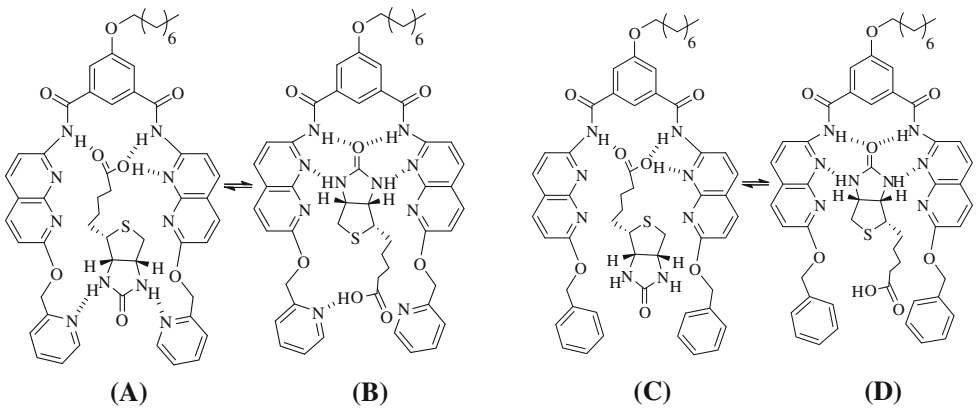


Fig. 6 Possible modes of complexation of biotin with **1** (**A** and **B**) and **2** (**C** and **D**)

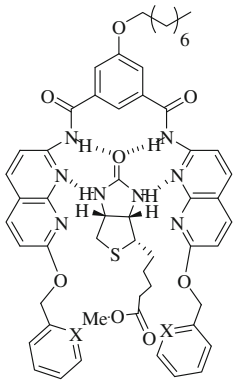


Fig. 7 Possible mode of complexation of biotin methyl ester with **1** and **2**

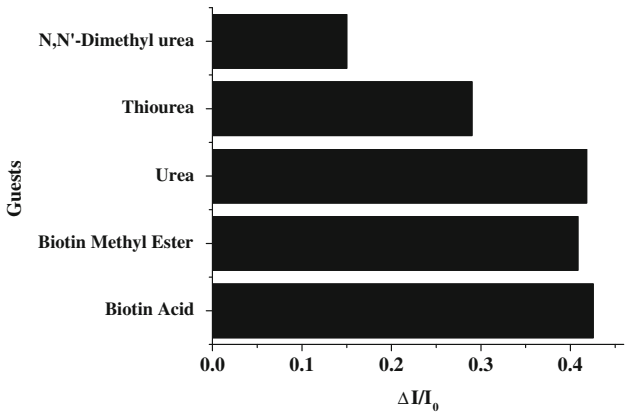


Fig. 8 Fluorescence ratio ($I\text{-}I_0/I_0$) of receptor **1** ($c = 1.49 \times 10^{-5}$ M) at 360 nm upon addition of 7 equiv of a particular guest in $CHCl_3$ containing 1% CH_3CN

Fluorescence and UV studies

The effect on absorption and emission spectra of naphthyridine-based receptors **1** and **2** was recorded in presence of urea, biotin and their derivatives in $CHCl_3$ containing 1% CH_3CN. Receptor **1** ($c = 1.49 \times 10^{-5}$ M) showed an emission at 360 nm when excited at 320 nm in $CHCl_3$ containing 1% CH_3CN. Upon gradual addition of biotin, biotin methyl ester, urea, thiourea and *N*, *N*′-dimethyl urea individually to the solution of receptor **1** in $CHCl_3$ containing 1% CH_3CN, the emission of naphthyridine was increased to the different extents (Fig. 8).

During titrations of receptor **1** with biotin, biotin methyl ester and urea, strong fluorescence enhancement effect was observed. For example, the spectral changes in fluorescence of **1** during titrations with biotin and urea are represented in Figs. 9a and 10a, respectively. Upon complexation of biotin and urea, the emission peak of **1** at 360 nm for naphthyridine underwent a red shift of 3 nm, suggesting a strong hydrogen bonding interaction. During titration no other spectral changes were observed in the emission spectra. The gradual increase of intensity of the monomer emission of naphthyridine motif during complexation is explained by hydrogen bond mediated perturbation of the excited $n\pi^*$ state in a destabilizing manner for which the $\pi\pi^*$ state becomes the lowest energy singlet excited state [26].

Simultaneous UV–vis titrations of **1** with the same guests were carried out in $CHCl_3$ containing 1% CH_3CN. The changes in absorbance of **1** upon complexation of biotin and urea are represented in Figs. 9b and 10b, respectively. Fluorescence titration data were used to determine the binding constant values [27]. For the complexes of **1** with biotin and urea, $I_0/\Delta I$ as function of the inverse of guest concentration fits a linear relationship, indicating 1:1 stoichiometry of receptor/guest complex. The ratio of the intercept versus slope gave the binding constant (K_a) value (see Supplementary Material).

As can be seen from Table 1, simple receptor **1** shows selectivity for biotin and urea. Urea, being smaller in size, is preferentially complexed than thiourea in the excited state. Weak binding of thiourea is attributed to the bigger size of the sulfur atom that poorly fits into the cavity than urea. The polarisability of the sulfur atom and charge transfer phenomena is also the responsible factors for the weaker hydrogen bond interaction of sulfur atom [28].

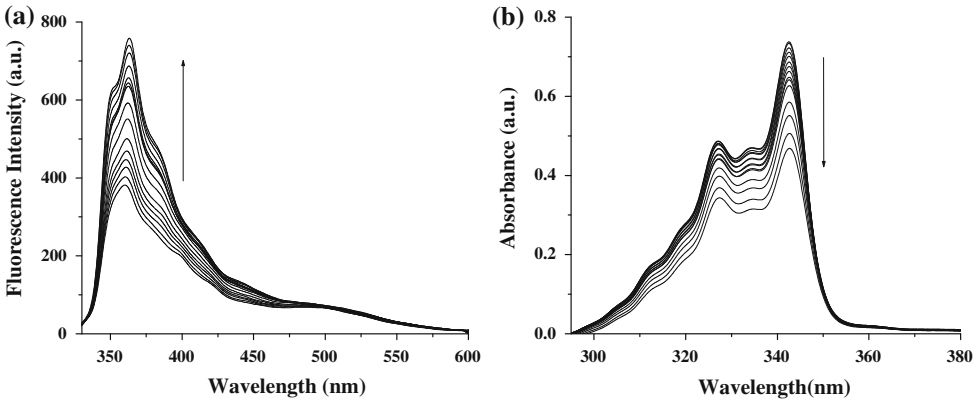


Fig. 9 (**a**) Change in emission of **1** ($c = 1.49 \times 10^{-5}$ M) and (**b**) change in absorbance of **1** ($c = 1.49 \times 10^{-5}$ M) upon gradual addition of biotin in $CHCl_3$ containing 1% CH_3CN

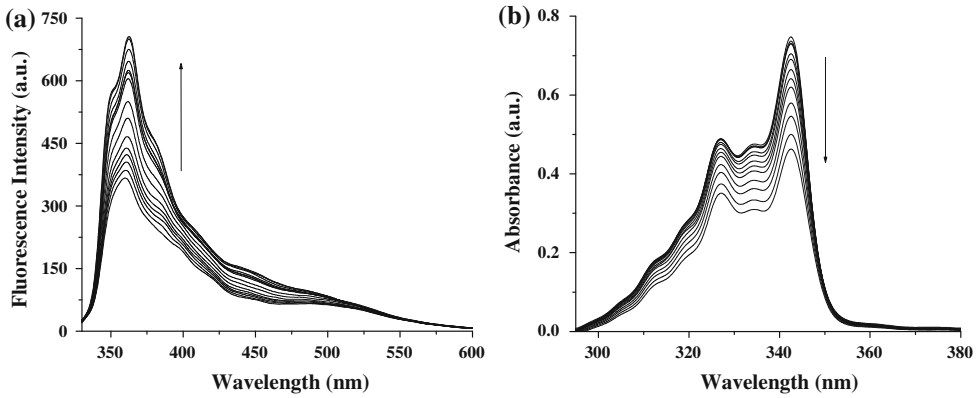


Fig. 10 (**a**) Change in emission of **1** ($c = 1.49 \times 10^{-5}$ M) and (**b**) change in absorbance of **1** ($c = 1.49 \times 10^{-5}$ M) upon gradual addition of urea in $CHCl_3$ containing 1% CH_3CN

Table 1 Binding constant values of **1** from fluorescence in $CHCl_3$ containing 1% CH_3CN

Guests	K_a (M^{-1})[a]
Biotin acid	1.08×10^4
Biotin methyl ester	8.2×10^3
Urea	1.02×10^4
Thiourea	b
N,N′-dimethyl urea	b

[a] Binding constant values were determined at wavelength 360 nm

[b] Binding constant values were not determined due to minor change

N,*N*′-dimethylurea is weakly complexed into the open cleft due to its bulky nature.

Under similar experimental conditions, titrations of receptor **2** were conducted in $CHCl_3$ containing 1% CH_3CN (see Supplementary Material). In contrast to receptor **1**, upon increasing amounts of addition of urea, biotin and their derivatives individually, to the receptor solution of **2**, minor change in fluorescence intensity of naphthyridine moiety was observed; suggesting weak interactions (also see explanation in ^{1}H NMR study). Due to the minor and irregular changes in emission of **2** upon complexation, reliable binding constant values were difficult to determine from fluorescence titration results. On the other hand, the absorbance at 343 nm gradually decreased upon addition of biotin, urea and their derivatives (Supplementary Material). The change in absorbance of the naphthyridine motif at 343 nm was considered for determination of binding constant and we were unable to determine satisfactorily. In such situation the dilution effect during the course of titration cannot be ignored.

In order to evaluate the sensing and binding ability of **3** as a receptor for urea, fluorescence studies were carried out in $CHCl_3$ containing 1% CH_3CN. Receptor **3** gave emission at 343 nm when excited at 310 nm. Addition of urea, thiourea or *N*,*N*′-dimethylurea individually to the solution of **3** caused marginal change in emission. Figure 11, for example, represents the change in emission and absorption spectra of **3** upon adding urea up to 15 equivalents.

Upon adding urea the absorptions of the bands at 310, 318 and 325 nm of **3** were decreased without showing any other spectral change. The similar change was also observed for thiourea and *N*,*N*′-dimethylurea. From the fluorescence and UV titrations data, it was difficult to determine the reliable association constant values for **3** with urea, thiourea or *N*,*N*′-dimethylurea.

Under similar experimental condition, **4** was also titrated with urea, thiourea and *N*,*N*′-dimethylurea in $CHCl_3$ containing 1% CH_3CN (see Supplementary Material). Here also we got similar findings as observed with the receptor

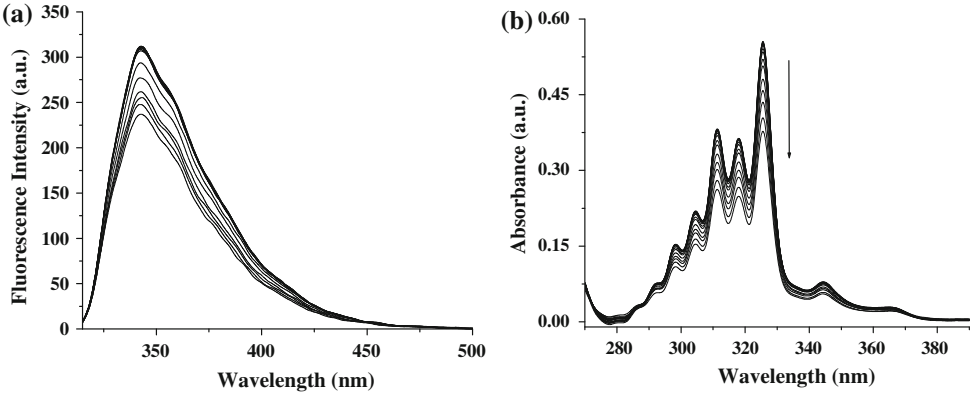


Fig. 11 (**a**) Emission spectra of **3** ($c = 2.44 \times 10^{-5}$ M) and (**b**) absorption spectra ($c = 2.44 \times 10^{-5}$ M) during titration with urea in $CHCl_3$ containing 1% CH_3CN

3. There was no appreciable change in emission of **4** upon adding urea or its derivatives. After the addition of 10 equivalent amounts of guests, the emission intensity at 343 nm (when excited at 310 nm) was gradually decreased. This decrease in emission intensity may occur due to dilution effect.

Thus the experimental results as corroborated above represent that only the receptor **1** is a better binder of urea and biotin due to the placement of the pyridyl ring at the lower rim.

In order to realize the complexing abilities of **1** and **2** with urea in less polar solvent $CHCl_3$, we further carried out the fluorescence and UV titration experiments by dilution of the 1:1 complexes of urea. Figure 12a, in this regard, indicates the change in absorbance of naphthyridine with **1**-urea complex concentration. The linear change in absorbance at 366 nm in **1** demonstrated 1:1 stoichiometry of the complex of **1** with urea (Fig. 12b).

A similar UV study with **2** gave linear change in absorbance at 343 nm, which indicated 1:1 stoichiometry of the complex of **2** with urea (Supplementary Material).

To understand the sensing property of receptors **1** and **2**, fluorescence titrations by dilution method were performed in the presence and absence of urea. It is evident from Fig. 13a, while emission at 363 nm in $CHCl_3$ is markedly increased or '*switched on*' with a red shift of 4 nm in presence of equivalent amount of urea to the solution of **1**, it is decreased in presence of thiourea. A similar observation was made with the receptor **2** in dry $CHCl_3$ (Fig. 13b). Figure 14a displays the change in emission spectra upon dilution of the complex of **1.urea**. The change in emission of the naphthyridine moiety at 366 nm for the 1:1 complex of **1** with urea is presented in Fig. 14b, showing an almost linear response with complex concentration. The same behavior was noticed with the urea complex of receptor **2** in $CHCl_3$ (see Supplementary Material).

Binding constant values were determined by dilution method [29] using UV titration results in dry $CHCl_3$ and receptor **1** shows about a 10 times higher binding constant value ($K_a = 8.73 \times 10^5$ M^{-1}) in $CHCl_3$ than the case in $CHCl_3$ containing 1% CH_3CN (*cf.* Table 1). On the other hand, receptor **2** exhibits a binding constant value of 1.15×10^5 M^{-1}, which is less than **1**. It is fact that CH_3CN, being more polar than $CHCl_3$ reduces the hydrogen bonding interactions, for which guest induced change in emission of **2** in comparison to **1**, in $CHCl_3$ containing 1% CH_3CN was too less to consider for determination of the binding constant values.

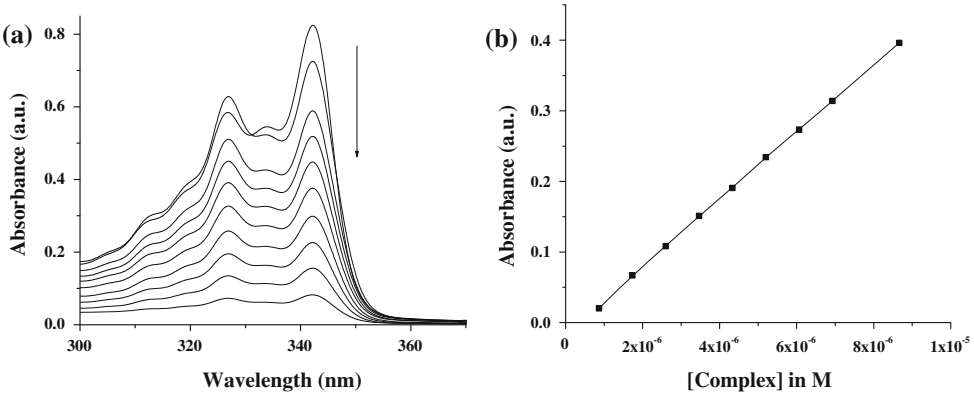


Fig. 12 (**a**) UV spectra of the complex **1**. urea ($c = 8.66 \times 10^{-6}$ M) and its change of absorbance on dilution; (**b**) plot of absorbance versus concentration of the 1:1 complex of urea with **1**

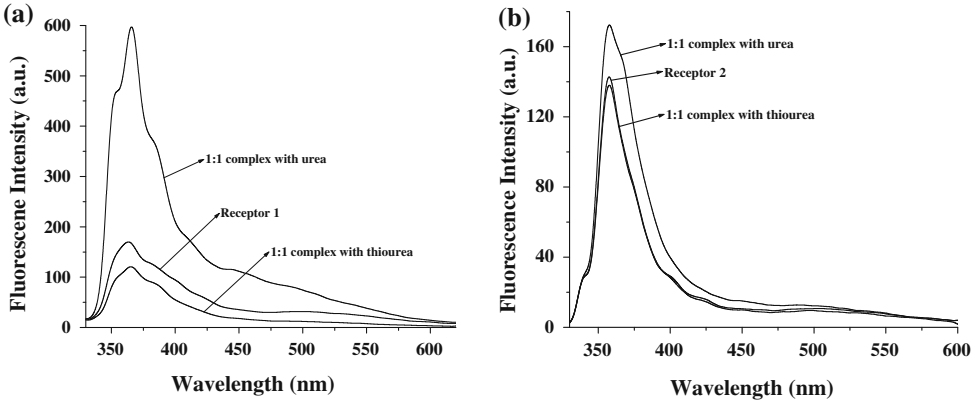


Fig. 13 (**a**) Emission spectra of **1** ($c = 8.66 \times 10^{-6}$ M) and its 1:1 complexes with urea and thiourea; (**b**) Emission spectra of **2** ($c = 1.26 \times 10^{-5}$ M) and its 1:1 complexes with urea and thiourea

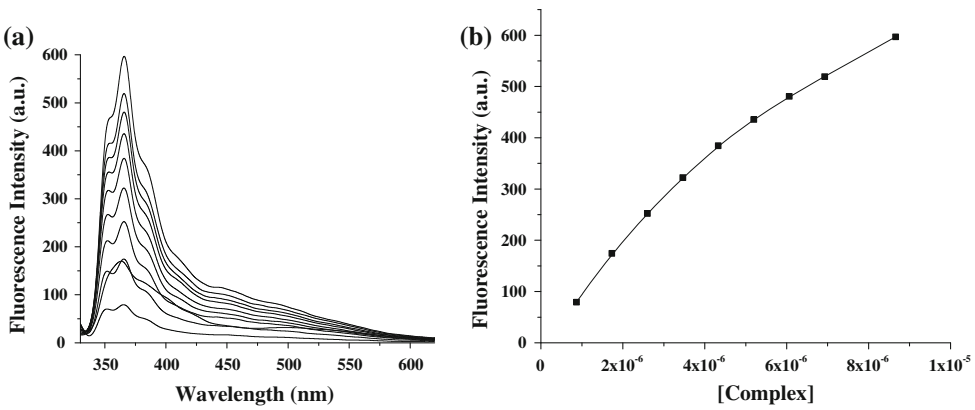


Fig. 14 (**a**) Change in emission of **1** ($c = 8.66 \times 10^{-6}$ M) in $CHCl_3$ in presence of stoichiometric amounts of urea and the dilution spectra of the 1:1 complex ($\lambda_{ex} = 320$ nm); (**b**) plot of fluorescence intensity versus concentration of the 1:1 complex of urea with **1**

Conclusions

We have designed and synthesized naphthyridine-based receptors as fluorescent chemosensors for detection and sensing of biologically important substrates biotin, urea and their derivatives in $CHCl_3$ containing 1% CH_3CN. The binding constant values as determined are moderate and appreciable. The emission of naphthyridine motif in receptor **1** effectively senses the complexation of biotin and urea in $CHCl_3$ containing 1% CH_3CN by exhibiting an increase in intensity during titration. It is also worth mentioning that the synergistic binding of carboxylic acid and urea motifs of biotin into the cleft of a naphthyridine-based receptor is necessary for effective sensing of biotin in solution. We conclude that due to this particular reason, receptor **1** is a better receptor for biotin than **2**. Binding experiments in dry $CHCl_3$ reveal that both the receptors **1** and **2** have the good affinity for urea. Future efforts will be aimed at defining the scope of the binding of such biologically important substrates by new fluorescent receptors with structural variations.

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