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Tryptophan receptors containing acridine-based thiourea

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Four derivatives of acridine and acridinium compounds (**L1**, **L2**, **L1H** and **L2H**) comprised thiourea-binding sites were synthesised. The binding abilities of receptors **L1**, **L2**, **L1H** and **L2H** towards amino acids (L-Trp, L-Phe, L-Leu, L-Ala and L-Gly) were studied by ^{1}H NMR spectroscopy, UV–vis and fluorescence spectrophotometry. Hydrogen bonding interactions between thiourea-binding site of the ligand and the carboxylate groups in zwitterionic amino acids were found to be the main interactions driving complexation to take place. The stoichiometry of 1:1 ligand to amino acid was observed in all cases. Neutral ligands **L1** and **L2** showed weak binding towards all studied amino acids. The cyclic ligand **L1** showed better binding ability towards tryptophan (Trp) than the acyclic ligand **L2** did (*K* for Trp is 307 and 266 M^{-1} for **L1** and **L2**, respectively). Interestingly, binding abilities of the protonated ligands, **L1H** and **L2H**, towards studied amino acids, especially Trp (*K* for Trp is 3157 and 2873 M^{-1} for **L1H** and **L2H**, respectively), were increased due to $R{-}COO^{-}\cdots H\cdots N^{+}$–acridinium interactions. Calculated structures of **L1H**·Trp and **L2H**·Trp showed that the polyglycol moiety in **L1H** provided a hydrophobic cavity for binding Trp resulting in a stronger binding affinity of **L1H** over **L2H**.

Keywords: acridine; acridinium; amino acid receptor; fluorescence sensor

Introduction

Molecular recognition of amino acids and their derivatives has been one of the most attractive objects in host–guest chemistry (*1–4*). It is partly due to the importance of amino acids as major components of proteins in natural living systems and partly due to their potential characteristics that make amino acids suitable for guest molecules in artificial recognition systems. Amino acids have versatile abilities to form complexes with other molecules via various kinds of intermolecular interactions (*5–7*). Ammonium and carboxylate groups in amino acids are expected to bind host molecules via hydrogen bonding and electrostatic interactions. Side chain of amino acids on the α-carbon can also form complexes with receptors using electrostatic interactions, van der Waals forces, hydrophobic interactions and π–π stacking interactions.

High sensitivity and abundance of fluorophores makes fluorescence spectrophotometry an ideal tool in developing biosensors (*8–11*). However, fluorescence sensors for amino acids are still rare. The binding of fluorophores with an aromatic amino acid such as tryptophan (Trp) causes quenching of the emission (*12*, *13*). Recently, many fluorescent supramolecular systems have been constructed and their abilities for amino acids recognition using non-covalent interactions have been investigated (*14–17*). A system of metal complexes and fluorescent indicators was used successfully in sensing amino acids (*18*, *19*). The changing in colour or switching on/off in fluorescence occurred from the displacement of the indicator by amino acids. Transformation of the ligand structure from a closed ring to an opened form after the binding of amino acids was also employed to obtain significant changes in fluorescence intensity (*20*, *21*).

We reported herein the syntheses of thiourea-containing acridine groups that are classified into two types: cyclic (**L1** and **L1H**) and acyclic receptors (**L2** and **L2H**). **L1H** and **L2H** are the protonated forms of **L1** and **L2**, respectively. We then examine the effects of cyclic or acyclic structure on the binding ability towards amino acids (L-Trp, L-Phe, L-Leu, L-Ala and L-Gly). **L1H** and **L2H** were expected to improve the binding properties with amino acids under the complementary interactions including electrostatic force and hydrogen bonding interactions.

Results and discussion

Design and synthesis

Acridine derivatives such as acridinium salt (*18*), acridinone (*19*, *20*) and acridinedione (*21*, *22*) have been used quite successfully in sensing strong basic anions. Our design is to use acridine as a signalling unit connecting to a thiourea receptor. König (*23*) and de Mendoza (*24*)

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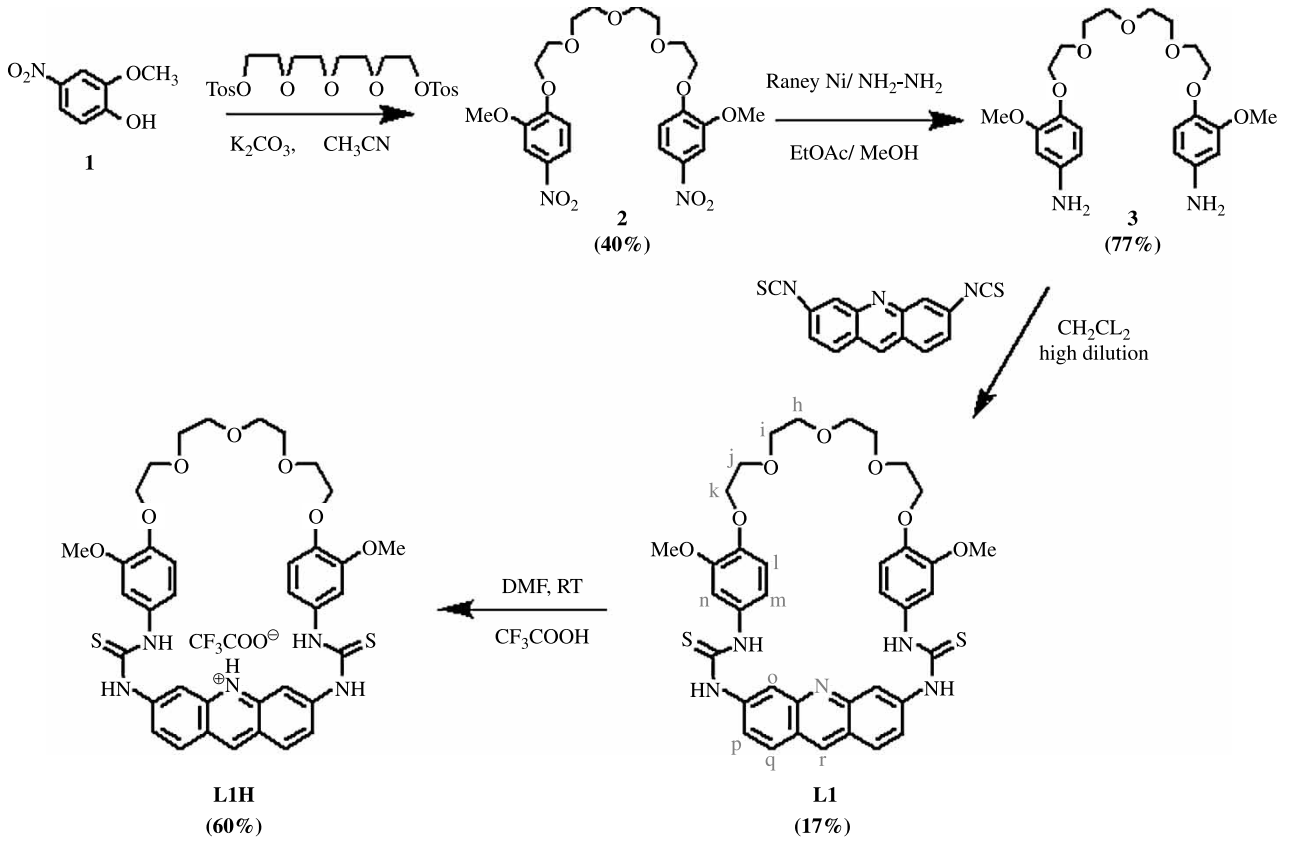
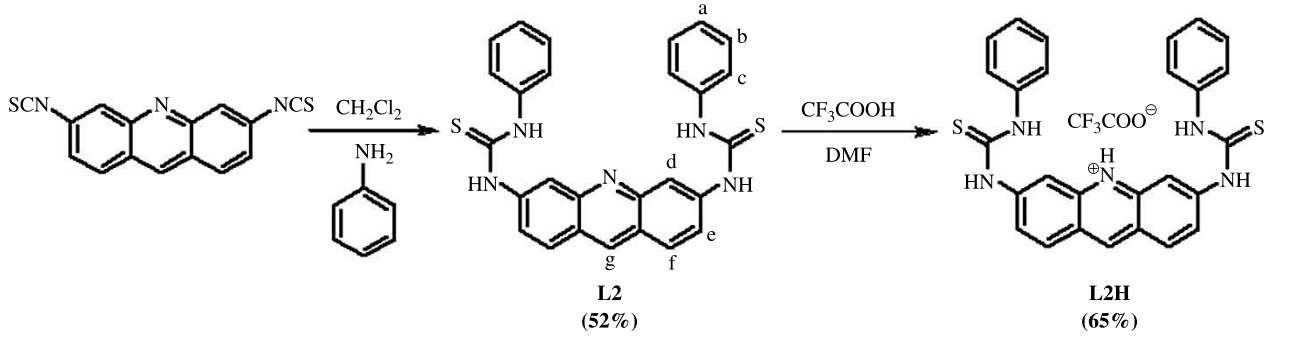


Scheme 1. Syntheses of receptors **L1** and **L1H**.

reported selective amino acid receptors containing crown ether moieties. We, therefore, incorporate a polyglycol unit into our desired ligand **L1**. A controlled ligand having no crown unit, **L2**, has also been synthesised to compare with **L1**. Both **L1** and **L2** were then protonated to produce **L1H** and **L2H**, respectively.

Syntheses of all receptors are summarised in Schemes 1 and 2. The synthesis of **L1** was started by a coupling reaction of compound **1** with tetraethylene glycol ditosylate in the presence of K_2CO_3 in CH_3CN giving the crown-like ether compound **2** in 40% yield. Diamino compound **3** was obtained in 77% yield by the reduction of compound **2** with Raney nickel and hydrazine. A coupling reaction of diamino **3** and proflavinedithioisocyanate produced the desired product (**L1**) as a red solid in 17% yield. The receptor **L2** was prepared in one step by a reaction of proflavinedithio-isocyanate and aniline to yield a pale orange powder in 52%. ^{1}H NMR spectra of **L1** and **L2** showed that the NH thiourea resonanced at downfield positions, 10.19



Scheme 2. Synthesis of receptors **L2** and **L2H**.

and 10.09 ppm for **L1** as well as 10.28 and 10.14 ppm for **L2**. The receptor **L1H** and **L2H** were obtained as a dark red powder and as a bright orange powder from reactions of **L1** and **L2**, respectively, with trifluoroacetic acid. ^{1}H NMR spectra of **L1H** and **L2H** showed that the NH thiourea and acridine protons appeared at farther downfield positions around 10.50–11.40 and 7.60–9.60 ppm for the aromatic region resulting from the protonation of *N*-acridine (*25*, *26*).

Complexation studies using ^{1}H NMR spectroscopy and UV–vis spectrophotometry

Binding properties of **L1** and **L2** with hydrophobic aliphatic amino acids (L-Leu, L-Ala and L-Gly) and hydrophobic aromatic amino acids (L-Trp and L-Phe) were first studied using ^{1}H NMR spectroscopy. Addition of excess amount of the amino acids to **L1** and **L2** in DMSO-d_6 resulted in remarkable downfield shifts of the N*H* thiourea resonances, particularly Trp and Phe (Figure 1).

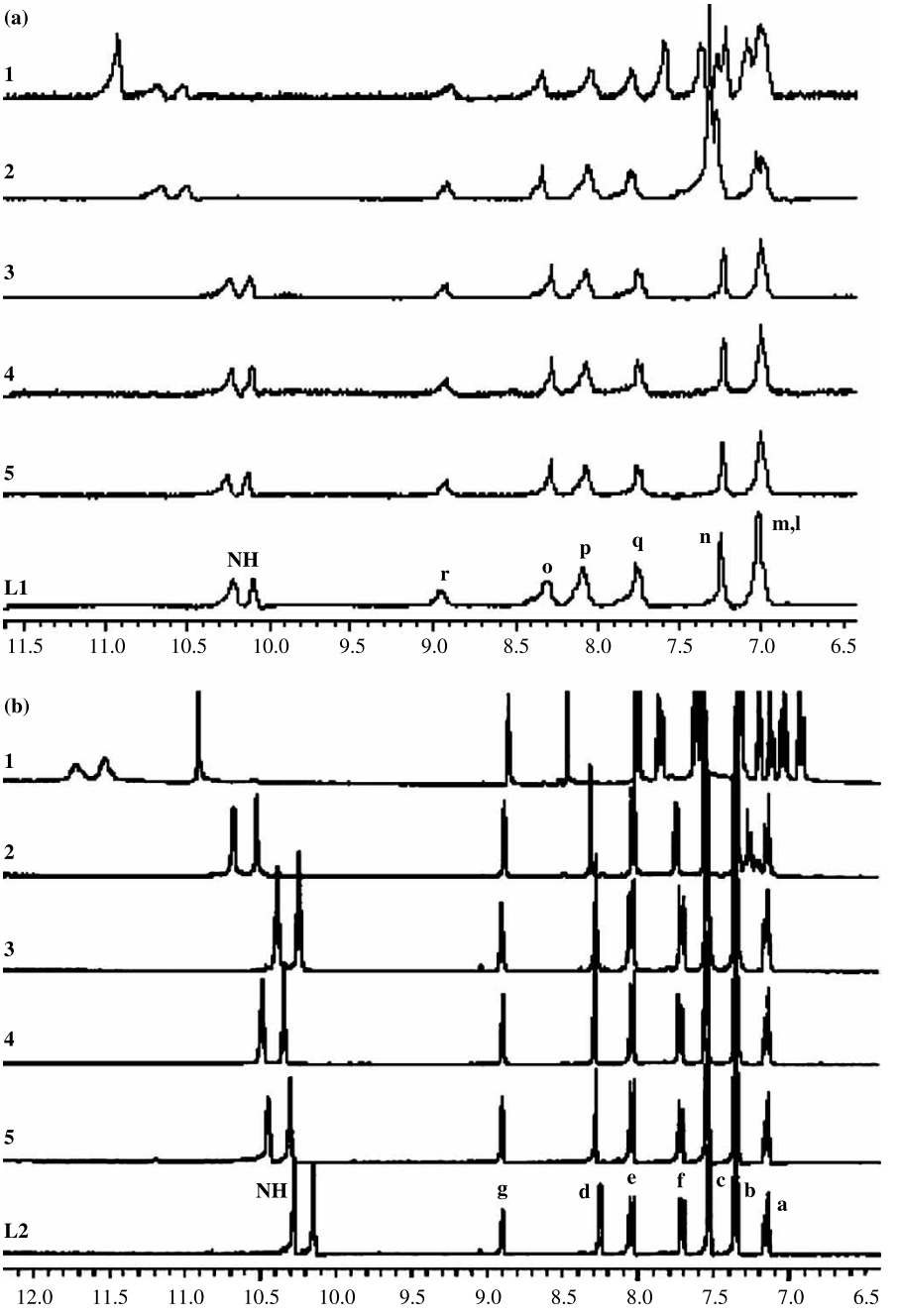


Figure 1. ^{1}H NMR (400 MHz) of (a) **L1** and (b) **L2** in DMSO-d_6 upon addition of excess amount of (1) Trp, (2) Phe, (3) Leu, (4) Ala and (5) Gly.

The complexes of both receptors and guests occurred via hydrogen bonding interactions between thiourea units on receptor and the carboxylate moiety of zwitterionic amino acids. The stoichiometry of the complexes of **L1** and **L2** with Trp and Phe was evaluated by the Job plot analysis monitoring the N*H* thiourea signals indicating a 1:1 ratio for the ligand and the guest.

Considering ^{1}H NMR spectra shown in Figure 2, the NH thiourea and acridinium protons of **L1H** exhibited upfield shifts upon addition of Trp. Phe and aliphatic amino acids caused slight shifts of the NH thiourea and acridinium protons. This is probably due to the higher p*K* (α-COOH) of Trp which enhances the interactions between the carboxylate group and NH^+-acridinium. Addition of Trp beyond 1 equivalent induced the downfield shift of the NH thiourea protons of **L1H** in a manner similar to that found in **L1**. This indicated that amino acids bound to the thiourea-binding site via H-bonding interactions. **L2H** also displayed changes in ^{1}H NMR spectra similar to those of **L1H** upon addition of the studied amino acids.

UV–vis spectra of **L1** and **L2** in the presence of amino acids in DMSO were recorded. The absorption or emission of acridine was derived from an inversion of two excited states: n–π* and π–π*. In aprotic solvents such as DMSO, the n–π* state is presumably the lowest state (*27*) resulting in the appearance of the maximum absorption band of both receptors at 400 nm. As shown in Figure 3(a), the band at 400 nm of **L1** shifted to 460 nm in the presence of amino acids. The similar result was found in the case of **L2** upon adding Trp. Other amino acids displayed a slight decrease in the absorption band at 400 nm without the appearance of a new band. Presumably, the new absorption band at 460 nm corresponded to the acridinium ion produced from the protonation at *N*-acridine by amino acids. This protonation stabilised π–π* excited state, caused the level inversion and resulted in the bathochromic shift of the absorption band.

The acridinium receptors **L1H** and **L2H** showed a maximum absorption band at 460 nm. The absorption band of **L1H** and **L2H** at 460 nm progressively decreased while a new band at 400 nm appeared with an isosbestic point at 415 nm upon amino acids. It was found that ligands **L1H** and **L2H** showed significant changes in UV–vis spectra upon addition of Trp. Addition of 10 equivalent of Trp induced the complete disappearance of the absorption band at 460 nm of **L1H** (Figure 3(b)), while the other amino acids needed more than 20 equivalent to suppress this band completely. However, **L2H** exhibited complete disappearance of the absorption band at 460 nm with 5 equivalent Trp and more than 10 equivalent of other amino acids.

^{1}H NMR and UV–vis studies showed that **L1** and **L2** formed complexes with amino acids, especially Trp due to H-bonding interactions only. The acridinium forms, **L1H** and **L2H**, employed both $R{-}COO^{-}\cdots H\cdots N^{+}$–acridinium interactions and H-bonding interactions. Therefore, **L1H** and **L2H** were expected to give higher binding affinity towards amino acids.

2D-NMR studies of L1·Trp and L2·Trp

In the light of ^{1}H NMR and UV–vis titrations, the structure of the complex **L1**·Trp was deduced from

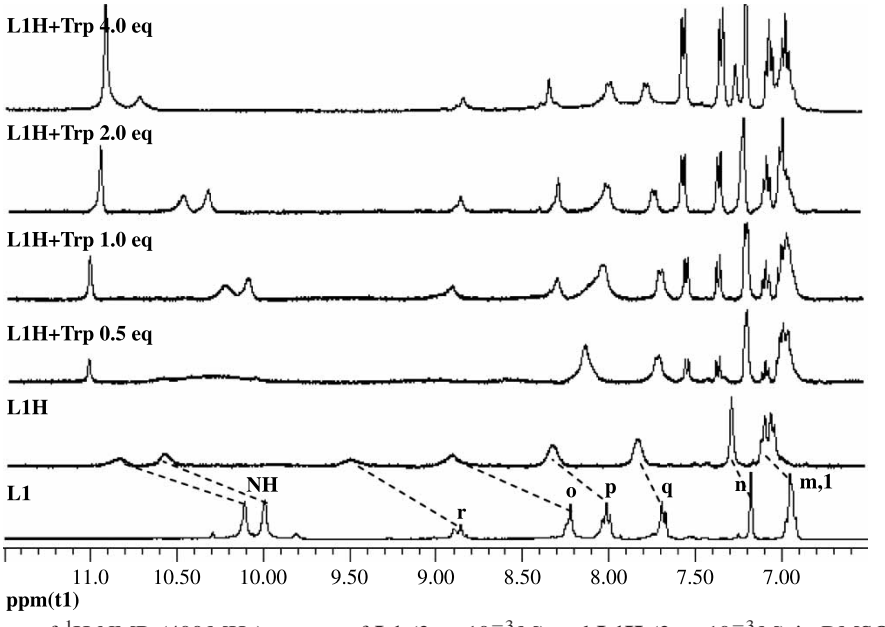


Figure 2. A comparison of ^{1}H NMR (400 MHz) spectra of **L1** (3×10^{-3} M) and **L1H** (3×10^{-3} M) in DMSO-d_6 upon addition of Trp 0.5, 1, 2 and 4 equivalent.

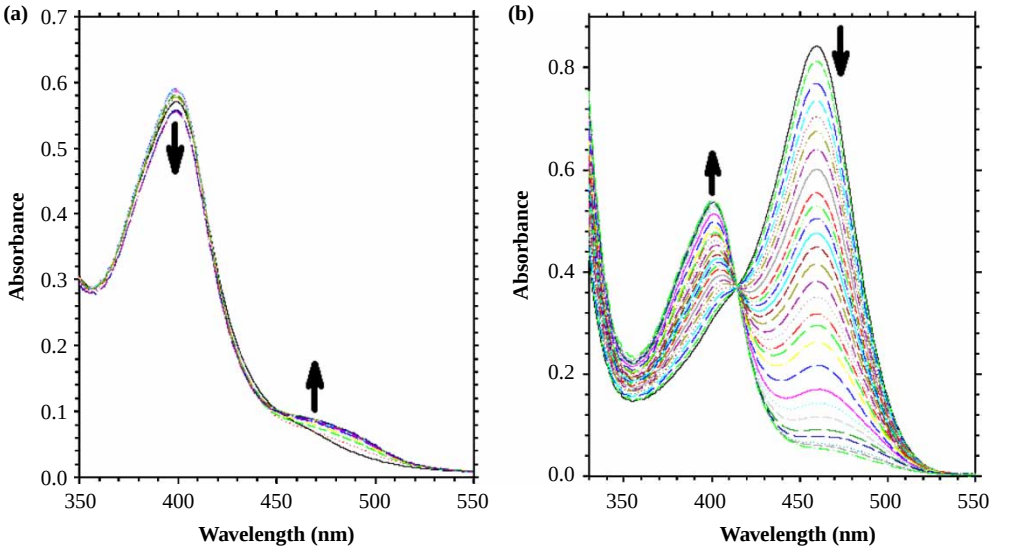


Figure 3. Changes in absorption spectra of (a) **L1** (2.5×10^{-5} M) upon addition of Trp 40 equivalent and (b) **L1H** (2.5×10^{-5} M) upon addition of Trp 30 equivalent.

NOESY. Correlation peaks between glycolic protons H_h, H_i and H_j of **L1** and aromatic protons H_s, H_u and H_t of Trp were observed. However, cross-relation peaks of acridine protons and Trp aromatic side-chain protons were not found. The proposed structure of the complex **L1**·Trp is shown in Figure 4.

In the case of **L2**, acridine protons H_d and H_f shifted downfield, while protons H_e and H_g shifted slightly upfield upon adding Trp. However, there are almost no changes in the protons of Trp. In addition, a NOESY spectrum of the complex **L2**·Trp did not show cross relationship of **L2** and Trp. The results implied that the existence of the polyethylene glycol unit in **L1** increased the interactions between **L1** and Trp.

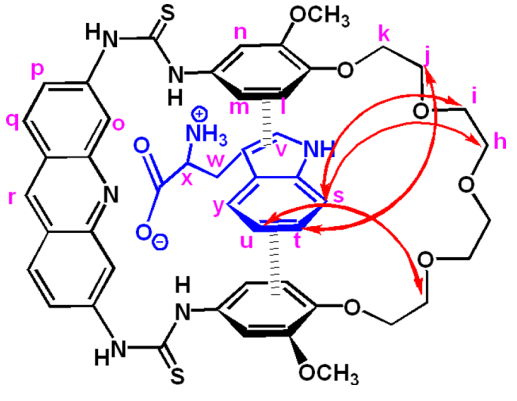


Figure 4. The proposed structure of **L1**·Trp deduced from NOESY cross relationship of **L1** and Trp.

Molecular modelling for L1H·Trp and L2H·Trp complexes

Figure 5 shows optimised structures of **L1H**·Trp and **L2H**·Trp. The calculated structures are consistent with the structures of **L1**·Trp and **L2**·Trp deduced from NOESY. Besides H-bonding interactions between the carboxylate unit and acridine protons, the macrocycle **L1H** provided a hydrophobic cavity for accommodating Trp. Weak interactions of an aryl ring proton of Trp and *O*-glycol of **L1H**, as well as $RNH_3^+ - \pi$ interactions were also observed. Moreover, the methoxy proton (H_{MeO}) of the host **L1H** showed weak interactions with the imidazole ring of Trp.

In contrast to **L1H**, **L2H** does not possess the polyethylene glycol unit. The supramolecular interactions with Trp guest relied on weak $RNH_3^+ - \pi$ interactions. Therefore, the binding affinity of **L2H** towards Trp is not as strong as that of **L1H**.

Complexation studies using fluorescence spectrophotometry

Fluorescence spectrometry was used to determine binding affinities of the synthesised hosts towards amino acid guests. The mechanism for sensing can operate via photoinduced electron transfer (PET) (*28–30*) and intramolecular charge transfer (*31–34*). Fluorescence emission change of **L1** (Figure 6(a)) and **L2** showed a small enhancement of emission bands at 528 and 520 nm, respectively, upon adding aromatic and aliphatic amino acids, but a large enhancement was observed for aromatic amino acid (Trp and Phe). The non-emissive via PET

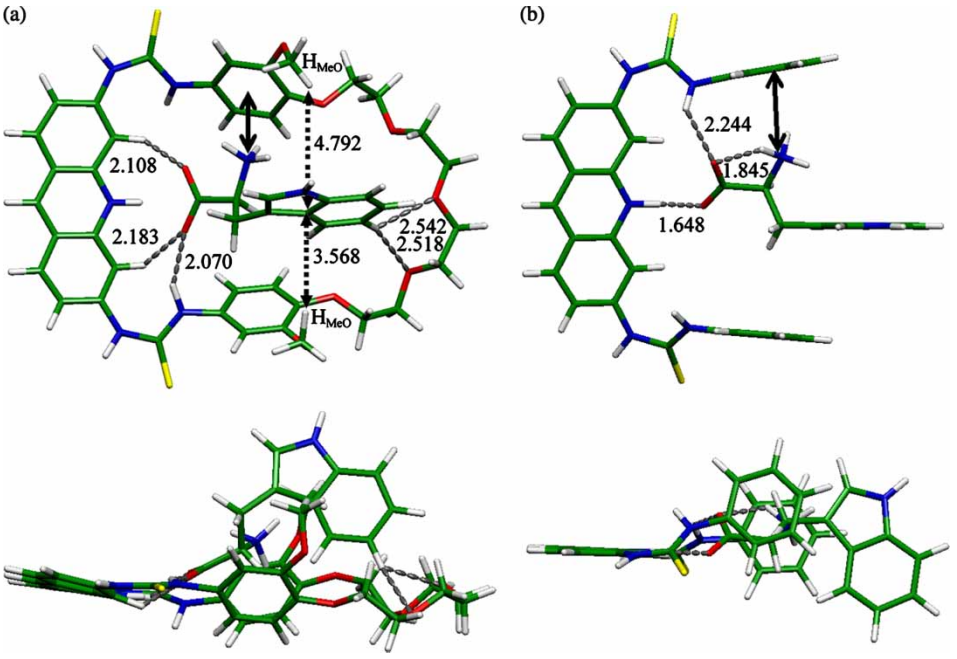


Figure 5. Top and side view optimised structures of the complexes of (a) **L1H** and (b) **L2H** and Trp. Bond distances shown are in Å.

mechanism of the unbound **L1** and **L2** may be ascribed to the electron-donating ability of the heteroatom in acridine leading to the electron transfer quenching pathway. For complexes of **L1** and **L2** with amino acids, this quenching pathway was eliminated possibly due to the protonation on N-atom of acridine by amino acids. Tables 1 and 2 summarise the binding constants (K) and the fluorescence enhancement (FE) factor, respectively, of all receptors in the presence of various amino acids. Data in both tables showed that the FEs of **L1** with aromatic amino acids are higher than those of **L1** with aliphatic amino acids. Therefore, the rigidified **L1** containing the glycolic ether

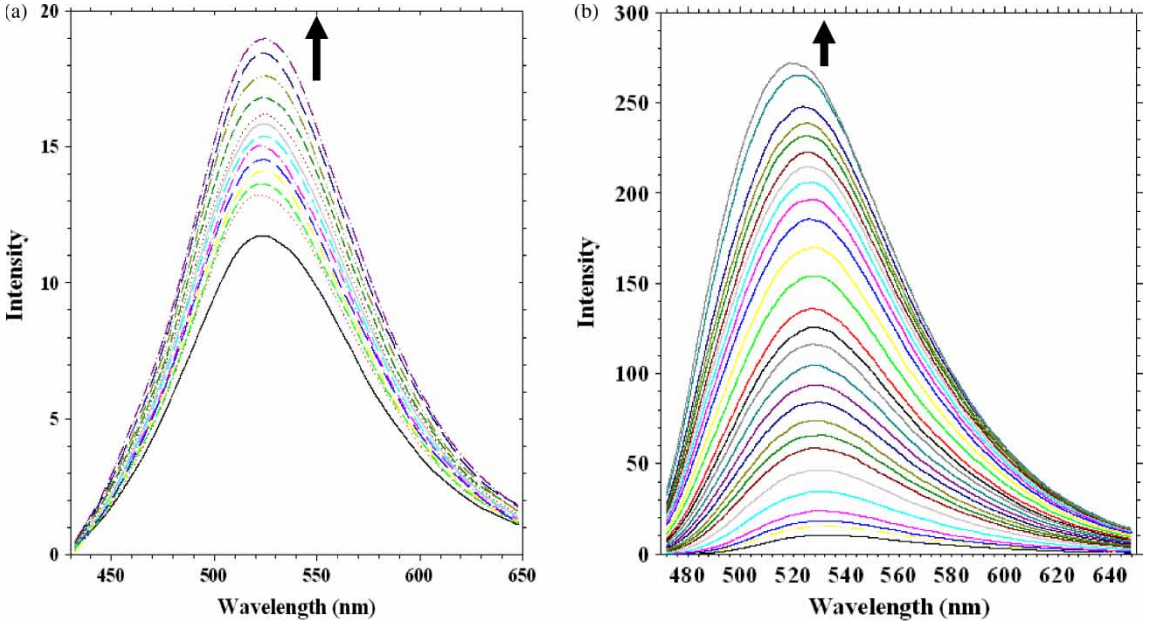


Figure 6. Changes in emission spectra of (a) **L1** (2×10^{-4} M, excitation at 400 nm) upon addition of Trp 30 equivalent in DMSO using $[Bu_4N]PF_6$ as a supporting electrolyte and (b) **L1H** (2×10^{-4} M, excitation at 460 nm) upon addition of Phe 25 equivalent in DMSO using $[Bu_4N]PF_6$ as a supporting electrolyte.

Table 1. The binding constants of interaction between **L1**, **L1H**, **L2** and **L2H** amino acids calculated from fluorescence titration using Benesi–Hildebrand equation (*38*).

	K				
	Trp	Phe	Leu	Ala	Gly
L1	307	578	*a*	219	*a*
L1H	3157	2748	855	900	957
L2	266	*a*	*a*	*a*	*a*
L2H	2873	2365	1418	1483	1367

a, values cannot be calculated.

bound more tightly with aromatic amino acids. On the contrary, there is no significant difference in FE values of **L2** with all amino acids.

The emission band of **L1H** was observed at 545 nm. This band undergoes a small bathochromic shift (17 nm in comparison with emission band of **L1**). This red shift is presumably due to the inversion from n–π* to π–π* excited state (*27*, *35*, *36*). The fluorescence intensity of **L1H** at 545 nm was gradually enhanced upon addition of Trp (Figure 6(b)). There was also a shift of emission band from 545 to 528 nm. Similar emission spectral changes were obtained for other amino acids. Upon addition of amino acids to **L2H**, the enhancement of emission band at 535 nm and the blue shift from 535 to 520 nm were observed. All amino acids gave the same results, except for Trp, which induced a higher fluorescent enhancement in both receptors. From Tables 1 and 2, **L1H** and **L2H** showed higher binding constants and FE values than **L1** and **L2**. This suggested that protonated hosts improved the binding abilities towards amino acids. The p*K* (α-COOH) values of Trp, Phe, Leu, Ala and Gly are 2.83, 1.83, 2.36, 2.34 and 2.34, respectively (*37*). These p*K* values are not significantly different. However, binding constants obtained from fluorescence titrations varied significantly among aromatic and aliphatic amino acids. Therefore, binding enhancement of **L1H** and **L2H** can be attributed to cooperation between $R—COO^{-}\cdots H\cdots N^{+}$–acridinium interactions and H-bonding interactions. Besides, other supramolecular interactions suggested by 2D NMR and modelling studies also contributed to the binding abilities of **L1H** and **L2H** towards studied amino acids, especially Trp.

Table 2. FE of receptors **L1**, **L1H**, **L2** and **L2H** with several guests[a].

	FE				
	Trp	Phe	Leu	Ala	Gly
L1	3.12	2.42	1.22	1.77	1.73
L1H	34.42	31.43	27.42	26.13	22.52
L2	1.53	1.42	1.29	1.35	1.28
L2H	24.17	20.98	18.86	17.55	17.99

[a] FE values were calculated as the ratio of fluorescence intensity in the presence and in the absence of guests.

Conclusion

We have synthesised four derivatives of acridine and acridinium compounds (**L1**, **L2**, **L1H** and **L2H**) containing thiourea-binding sites. Binding abilities of receptors **L1**, **L2**, **L1H** and **L2H** towards amino acids (Trp, Phe, Leu, Ala and Gly) were studied by ^{1}H NMR spectroscopy, UV–vis and fluorescence spectrophotometry, and showed that the preorganised host **L1** could bind selectively to aromatic amino acid such as Trp. Hydrogen bonding interactions between thiourea-binding sites of the ligands and carboxylate groups in zwitterionic amino acids were found to be the main interactions driving complexation to take place. Protonated hosts **L1H** and **L2H** showed the interactions of carboxylate group of Trp and NH^{+}-acridinium, which improved the binding ability of both ligands towards amino acids. Calculated structures of **L1H**·Trp and **L2H**·Trp showed that the glycolic unit in **L1H** provided the hydrophobic cavity for binding Trp (with additional supramolecular interactions) resulting in a stronger binding affinity of **L1H** over **L2H**.

Experimental

Materials and spectroscopic measurements

All materials were of standard analytical grade, purchased from Fluka, Aldrich or Merck and used without further purification. UV absorption spectra were obtained at 25°C on a Varian Cary 50 Probe UV–vis spectrophotometer. Fluorescence emission spectra were obtained on a Varian Cary Eclipse Fluorescence spectrophotometer. The solvent (DMSO) was of spectroscopic grade and used without further purification. Samples were contained in 10 mm path length quartz cuvettes (3.5 ml volume). Tetrabutylammonium hexafluorophosphate was used as a supporting electrolyte. NMR spectra were recorded in DMSO-d_6 on Variance 400 MHz spectrometer using tetramethylsilane as an internal standard.

Synthesis of L1, L2, L1H and L2H

*Synthesis of 2-methoxy-1-(2-(2-(2-(2-(2-methoxy-4-nitrophenoxy)ethoxy)ethoxy)ethoxy) ethoxy)-4-nitrobenzene (**2**)*

A mixture of **1** (0.2011 g, 0.0012 mol), catalytic amount of tetrabutylammonium bromide and potassium carbonate (1.6530 g, 0.012 mol) in 80 ml acetonitrile, was stirred at room temperature for 30 min. Then, a solution of tetraethylene glycol ditosylate (0.3010 g, 0.006 mol) in acetonitrile was added and the reaction mixture was heated under nitrogen atmosphere for 5 days. After the mixture cooled down to room temperature, the solvent was removed to dryness *in vacuo*. The residue was dissolved in dichloromethane and then washed with 3 M hydrochloric acid (50 ml) and extracted with dichloromethane

(3 × 50 ml). The organic phase was dried over anhydrous sodium sulphate and the solvent was removed. The desired product (**2**) was obtained as a bright yellow crystalline solid (40% yield) after recrystallisation in dichloromethane/methanol. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.916 (2H, dd, $J = 2.8$, 2.4 Hz), 7.765 (2H, d, $J = 2.4$ Hz), 6.985 (2H, d, $J = 8.8$ Hz), 4.308 (4H, t, $J = 4.8$ Hz), 3.966 (10H, s), 3.766 (4H, m, $J = 2.8$ Hz), 3.710 (4H, m, $J = 2.8$ Hz). IR (KBr, cm^{-1}): 3443, 3093, 2898, 1590, 1520, 1334, 1275, 1139, 1092. Anal. calcd for $C_{32}H_{28}N_2O_{11}$: C, 53.22; H, 5.68; N, 5.64; found: C, 53.22; H, 5.63; N, 5.74.

*Synthesis of 4-(2-(2-(2-(2-(4-amino-2-methoxyphenoxy)-ethoxy)ethoxy)ethoxy)ethoxy)-3-methoxybenzenamine (**3**)*

Hydrazine (0.4876 g, 0.021 mol) and Raney nickel (0.3073 g) was added to a vigorously stirred solution of **2** (0.2095 g, 0.004 mol) in 60 ml of 1:1 ethyl acetate:methanol. After 2 h, the mixture was filtered to remove Raney nickel and the filtrate was freed of solvent. The residue was dissolved in dichloromethane and dried over sodium sulphate anhydrous. Evaporation of the solvent gave the product **3**, as a yellow liquid in 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.76 (2H, dd, $J = 6.4$, 2.4 Hz), 6.25 (2H, s), 6.14 (2H, dd, $J = 6.2$, 2.4), 4.04 (4H, m), 3.90 (4H, s), 3.80 (4H, m), 3.74 (3H, s), 3.69 (4H, m), 3.63 (4H, m).

*Synthesis of **L1***

A solution of proflavinedithioisocyanate (1.1677 g, 0.004 mol) in dichloromethane was slowly added to the solution of **3** with the controlled temperature at 0°C and the reaction was stirred at room temperature overnight. The reaction mixture was filtered to remove dimer product as a dark red powder and then the organic solvent was evaporated under reduced pressure. The residue was dissolved in DMF and then methanol was added to precipitate the desired product **L1** as an orange powder (16% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.092 (2H, d, $J = 7.6$ Hz), 9.975 (2H, d, $J = 6.0$ Hz), 8.855 (1H, d, $J = 12.0$ Hz), 8.234 (2H, s), 8.001 (2H, d, $J = 8.0$ Hz), 7.680 (2H, d, $J = 9.6$ Hz), 7.181 (2H, s), 6.954 (4H, s), 4.041 (4H, s), 3.727 (10H, s), 3.571 (8H, d, $J = 7.6$ Hz). ^{13}C{^{1}H} NMR (400 MHz, DMSO-d_6): δ 179.4, 149.2, 148.6, 145.4, 141.6, 135.1, 132.5, 128.3, 123.3, 123.0, 117.6, 116.3, 113.2, 109.3, 69.9, 69.8, 68.9, 68.1, 55.6. IR (KBr, cm^{-1}): 3338, 2921, 2867, 1602, 1513, 1458, 1225, 1131. MS (ESI): *m/z* for $C_{37}H_{39}N_5O_7S_2$ 729.23 [$M^+ - H$]. Anal. calcd for $C_{37}H_{39}N_5O_7S_2{\cdot}0.6\ CH_2Cl_2{\cdot}0.4$ DMF: C, 57.47; H, 5.31; N, 9.33; found: C, 57.14; H, 5.32; N, 9.60.

*Synthesis **L1H***

To a solution of **L1** (0.0031 g, 0.0405 mmol) in DMF, an excess amount of trifluoroacetic acid (0.1000 ml) was added and then stirred at room temperature for 2 days. The desired product (**L1H**) was obtained as an orange solid in 60% yield after an addition of methanol to the reaction mixture. The final product (**L1H**) was obtained as a dark red solid in 60% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.532 (2H, s), 10.821 (2H, s), 10.547 (2H, s), 9.413 (1H, s), 8.848 (2H, s), 8.230 (2H, s), 7.738 (2H, s), 7.199 (2H, s), 7.003 (2H, s), 6.966 (2H, s), 4.055 (4H, s), 3.731 (10H, s), 3.563 (8H, d, $J = 8.4$ Hz). IR (KBr, cm^{-1}) 3529, 2918, 2867, 1676, 1509, 1458, 1201, 1123. MS (ESI): *m/z* for $C_{39}H_{40}F_3N_5O_9S_2$ 844.864 [$M + H^+$].

*Synthesis of **L2***

To a solution of aniline (0.1026 ml, 0.0011 mol) in 30 ml dichloromethane was cooled to 0°C and slowly added with a solution of proflavinedithioisocyanate (0.1500 g, 0.0005 mol) in dichloromethane. After the reaction mixture was stirred at room temperature overnight, a yellow solid was precipitated and the solid was filtered under vacuum and washed with dichloromethane to yield a pale orange powder as the final product (**L2**; 52% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.284 (2H, s), 10.144 (2H, s), 8.894 (1H, s), 8.251 (2H, s), 8.037 (2H, d, $J = 8.8$ Hz), 7.697 (2H, dd, $J = 8.8$, 3 Hz), 7.526 (4H, d, $J = 7.2$ Hz), 7.350 (4H, m, $J = 7.6$ Hz), 7.142 (2H, m). ^{13}C{^{1}H} NMR (400 MHz, DMSO-d_6): δ 179.6, 149.3, 142.1, 139.8, 135.4, 129.2, 124.8, 124.0, 123.8, 123.5, 118.0. IR (KBr, cm^{-1}) 3217, 3027, 1618, 1544, 1446, 1252, 1147. Anal. calcd for $C_{27}H_{21}N_5S_2$: C, 67.61; H, 4.41; N, 14.60; found: C, 67.59; H, 4.42; N, 14.61.

*Synthesis of **L2H***

To a solution of **L2** (0.0031 g, 0.0405 mmol) in DMF, an excess amount of trifluoroacetic acid (0.1000 ml) was added and then stirred at room temperature for 2 days. The desired product (**L2H**) was obtained as an orange solid in 65% yield after an addition of methanol to the reaction mixture. The final product (**L2H**) was obtained as a dark red solid in 65% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.085 (2H, s), 10.804 (2H, s), 9.453 (1H, s), 8.885 (2H, s), 8.274 (2H, m), 7.784 (2H, d, $J = 9.2$ Hz), 7.545 (4H, d, $J = 7.6$ Hz), 7.364 (4H, t, $J = 8.0$ Hz), 7.171 (4H, t, $J = 7.2$ Hz). ^{13}C{^{1}H} NMR (400 MHz, DMSO-d_6): δ 179.3, 159.7, 159.4, 159.0, 139.3, 130.8, 129.0, 125.6, 124.4, 123.3, 122.1, 118.9. IR (KBr, cm^{-1}) 3478, 3050, 2781, 1696, 1536, 1458, 1193. MS (ESI): *m/z* for $C_{32}H_{29}F_3N_6O_3S_2$ 665.752 [M^+ + DMF].

UV–vis titrations

UV–vis titration of all receptors (in a range of 2×10^{-5}–3×10^{-5} M) in the presence of amino acids were recorded in DMSO (using $[Bu_4N][PF_6]$ as a supporting

electrolyte). A solution of amino acids was prepared in spectroscopic grade DMSO and then it was introduced in portions (total volume 1.5 ml) into a solution of receptor (2 ml). Absorption spectra of solution were recorded after each addition until absorbance of a new peak (at 460 and 400 nm for neutral and protonated receptors, respectively) was constant.

Fluorescence titrations

Fluorescence titration of all receptors (2×10^{-4} M) in the presence of amino acids were recorded in DMSO (using $[Bu_4N][PF_6]$ as a supporting electrolyte). A solution of amino acids was prepared in spectroscopic grade DMSO and then it was introduced in portions (total volume 1.5 ml) into a solution of receptor (2 ml). Fluorescence intensity changes were recorded for all titrations. The neutral receptors (**L1** and **L2**) and the protonated receptors (**L1H** and **L2H**) were excited at 400 and 460 nm, respectively. Binding constants were calculated from fluorimetric data using Benesi–Hildebrand equation (*38*). The quantity $I_F^0/(I_F - I_F^0)$ is plotted versus $[M]^{-1}$, and the binding constants is given by the ratio intercept/slope.

Computation methods

Full geometry optimisation of complexes of **L1H** and **L2H** and Trp were computed by density functional theory using the Becke's three-parameter hybrid density function and the Lee, Yang, and Parr correlation function (B3LYP) (*39*, *40*) with the 6–31+G(d,p) basis set. All calculations were performed using GAUSSIAN 03 program (*41*).

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