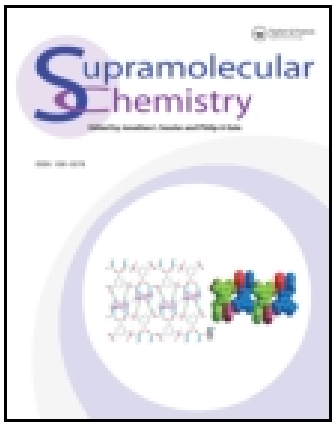

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Molecular recognition of ω-amino acids by thiazolobenzocrown receptors: a GABA-selective ionophore

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Molecular recognition of ω-amino acids by thiazolobenzocrown receptors: a GABA-selective ionophore

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Three new thiazolobenzocrown (TBC) ethers conjugated with picolinic acid, benzoic acid and pyridylmethyl were synthesised and their binding properties towards amino acids were assessed by ^{1}H NMR titration and isothermal titration calorimety (ITC). The TBC-picolinic acid conjugate (*4*) showed a pronounced selectivity towards GABA by the ^{1}H NMR titration, and the association constant for GABA had the largest value determined by ITC. The association constant of **4** for GABA was about 19 times higher than that of glycine.

Keywords: thiazolobenzocrown ether; picolinic acid; selective recognition; GABA; amino acid

1. Introduction

The development of artificial receptors for selective recognition of biologically interesting species has attracted attention because of their applications as molecular probes. Amino acids are the most important targets for molecular recognition by artificial host compounds. This is due to their relevance in the biological world and their rich biomimetic chemistry (*1*). For this reason, chemists have been studying host–guest binding of amino acids as an instrument for the manipulation of their reactivity (*2*). There have been several amino acid receptors reported based on crown ethers and heterocrown ethers (*3*), redox-active ferrocene unit (*4*), binaphthyl crown ether (*5*), calixarene (*6*), sapphyrin-lasalocid conjugates (*7*), macrobicyclic receptors (*8*) and macrocyclic metal complexes such as metalloporphyrins (*9*).

Recognition of amino acids by artificial receptors is limited due to the hydrophilic character of amino acids. In order to recognise the zwitterionic form of an amino acid effectively, simultaneous binding of ammonium and carboxylate groups is required.

Moreover, the electronic densities of carboxylate and ammonium groups are greatly affected by their mutual vicinity, causing the binding forces between the complementary groups and the receptor to be less effective for complexation (*10*). Thus, for the selective recognition of amino acids, convergent heteroditopic receptors that contain two different binding sites connected by a linker and capable of a simultaneous coordination with ammonium and carboxylate groups are required (*11*). However, the design of a convergent heteroditopic receptor is quite a challenge because the ion-binding sites have to be incorporated into a suitably preorganised scaffold that holds them in close proximity, but not so close that the sites interact. In most of the studies reported so far, crown ethers have been chosen as ammonium binding sites (*12–16*), and quaternary ammonium (*12–14*), guanidinium (*15–17*), urea (*17*) or metals (*18*, *19*) have been used as carboxylate binding sites.

γ-Aminobutyric acid (GABA), as one of two main inhibitory neurotransmitters, plays an important role in the mammalian central nervous system (*20*). The concentration of GABA in the brain is estimated to be approximately 1000 times that of the levels of classical monoamine neurotransmitters. More than one-third of the neurons in the brain use GABA for synaptic communication and the concentration of brain GABA essentially controls the mental and, to a large extent, the physical health of humans. It affects ionotropic and metabotropic receptors and altered GABAergic neurotransmission has been implicated in major neurological and psychiatric disorders such as anxiety and stress disorders, musculoskeletal and pain disorders, insomnia and sleep disorders, addiction and drug-withdrawal syndromes, epilepsy and seizures, brain ischaemia, mood disorders, schizophrenia, anaesthesia, liver diseases and hepatic encephalopathy, cognition, learning and memory disorders, premenstrual and other hormonal disorders and Alzheimer's disease (*21*). However, direct measurement of GABA concentrations in the brain faces several significant challenges, as it is difficult to detect GABA through an enzymatic reaction. In light of the ubiquitous importance of GABA to our health, the development of GABA receptors is of great therapeutic interest (*22*).

It is presumed that macrocyclic polyethers having two conformational features such as crown ether-type

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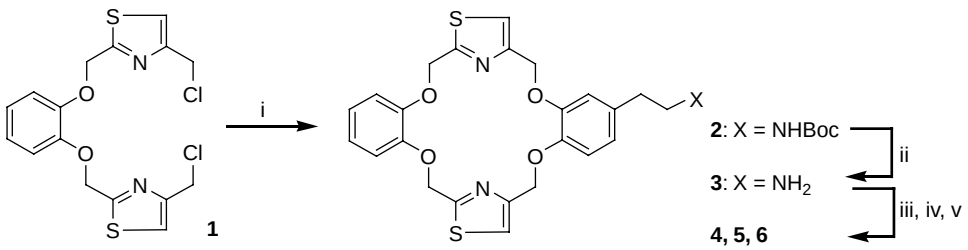

Scheme 1. (i) *N*-Boc-dopamine, NaI, K_2CO_3, butanone; (ii) CF_3CO_2H, CH_2Cl_2; (iii) picolinic acid, DIC, HOBt, CH_2Cl_2; (iv) benzoic acid, DCC, HOBt, CH_2Cl_2; (v) 2-(bromomethyl)pyridine, TEA, CH_2Cl_2.

compounds with right cavity size for NH_4^+ and those containing symmetrically positioned heterocylic units in the structure may exhibit enhanced NH_4^+ selectivity. It was reasoned that NH_4^+ may be nested in or perched on the cavity of a cyclic polyether and bound tightly via balanced hydrogen bonding to the ligand donor atoms of the heterocylic units (*23*). Thus, crown ethers that contain thiazole moieties have been reported to exhibit high ammonium (*23*), silver (*24*) and mercury ion selectivity (*25*). In this work, we used a dibenzo-18-crown-6 ether containing thiazole ring as an ammonium ion binding site, and for the first time, we used a conjugated picolinic amide as a carboxylate ion binding site. The new receptor, **4**, was synthesized, as shown in Scheme 1, and its binding affinity towards several amino acids, GABA and dipeptide was evaluated and compared with that of **5**, which has no pyridine moiety, and **6**, which has no amide bond (Figure 1).

2. Results and discussion

The synthesis of receptors **4**–**6** was carried out as shown in Scheme 1. The cyclisation of **1** with *N*-Boc-dopamine (*26*, *27*) in the presence of sodium iodide and K_2CO_3 resulted in **2** with a 72% yield, which was subsequently deprotected with trifluoroacetic acid resulting in free amine **3** with a 70% yield. This compound was converted into receptors **4**–**6** by coupling with the corresponding acid and 2-pyridylmethyl bromide. The reaction of **3** with picolinic acid and benzoic acid in the presence of 1,3-diisopropylcarbodiimide (DIC) and/or 1,3-dicyclohexylcarbodiimide (DCC), and 1-hydroxybenzotriazole (HOBT) in dichloromethane provided thiazolobenzocrown-picolinic acid conjugate (TBC-PAC) (*4*) and thiazolobenzocrown-benzoic acid conjugate (TBC-BAC) (*5*) with a 64 and 78% yield, respectively. The structure of TBC-PAC was confirmed by spectroscopic and analytical data. For instance, the ^{1}H NMR spectrum showed pyridine protons at δ 8.52, 8.05, 8.0 and 7.62, thiazole protons at δ 7.72 and 7.71, a broad amide proton at δ 8.29, and three sets of methylene protons at δ 5.24, 5.02 and 4.99, respectively. In addition, TBC-PAC receptor exhibited a characteristic amide absorption band at 1654 cm^{-1} in the IR spectrum. Similar structural assignments were made for TBC-BAC (*5*) and TBC-PMC (*6*), and the structure was the same as the proposed one.

An initial NMR screening of the binding affinities of **4** and its derivative **5** towards GABA showed a pronounced dependence of the association constant (K_a) on the nature of the aromatic group. In order to determine the selective binding ability of **4** towards an amino acid, we studied the ^{1}H NMR spectral changes caused by the addition of an amino acid to aqueous DMSO (3:7) solution containing the receptor. The ^{1}H NMR titration spectrum of the receptor **4** with GABA shows an interesting trend in their chemical shifts as the molar ratios of the ligand to NH_4^+ increase from 0 to 1 (Figure 2). When **4** was mixed with 1 equiv. of GABA, the proton signals of CO*NH* (H4), ThzCH_2O groups (H5), thiazolyl-*H* (H6) and pyridine-H_1 nearest to the nitrogen (H1) in **4** moved downfield. The proton signal of N-*H* and pyridine-H_1 clearly underwent a downfield shift from 8.26 to 8.43 ppm and from 8.58 to 8.66 ppm, respectively, due to the hydrogen bonding between the carboxylate anion and the amide N-*H* and acidic pyridine-H_1, which also indicates that the acidic proton of pyridine acts as a binding site of this heteroditopic receptor.

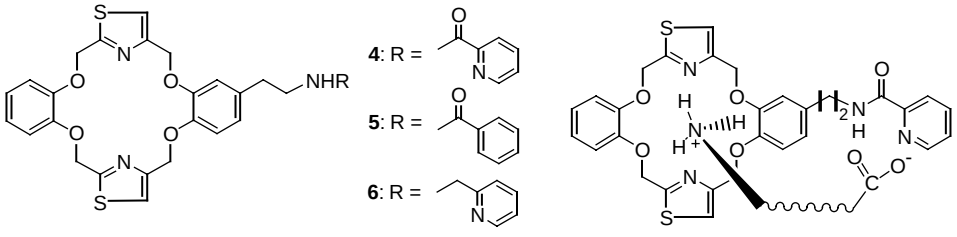

Figure 1. Structures of conjugates **4**–**6** and proposed structure of the complex for conjugate **4** with the amino acid.

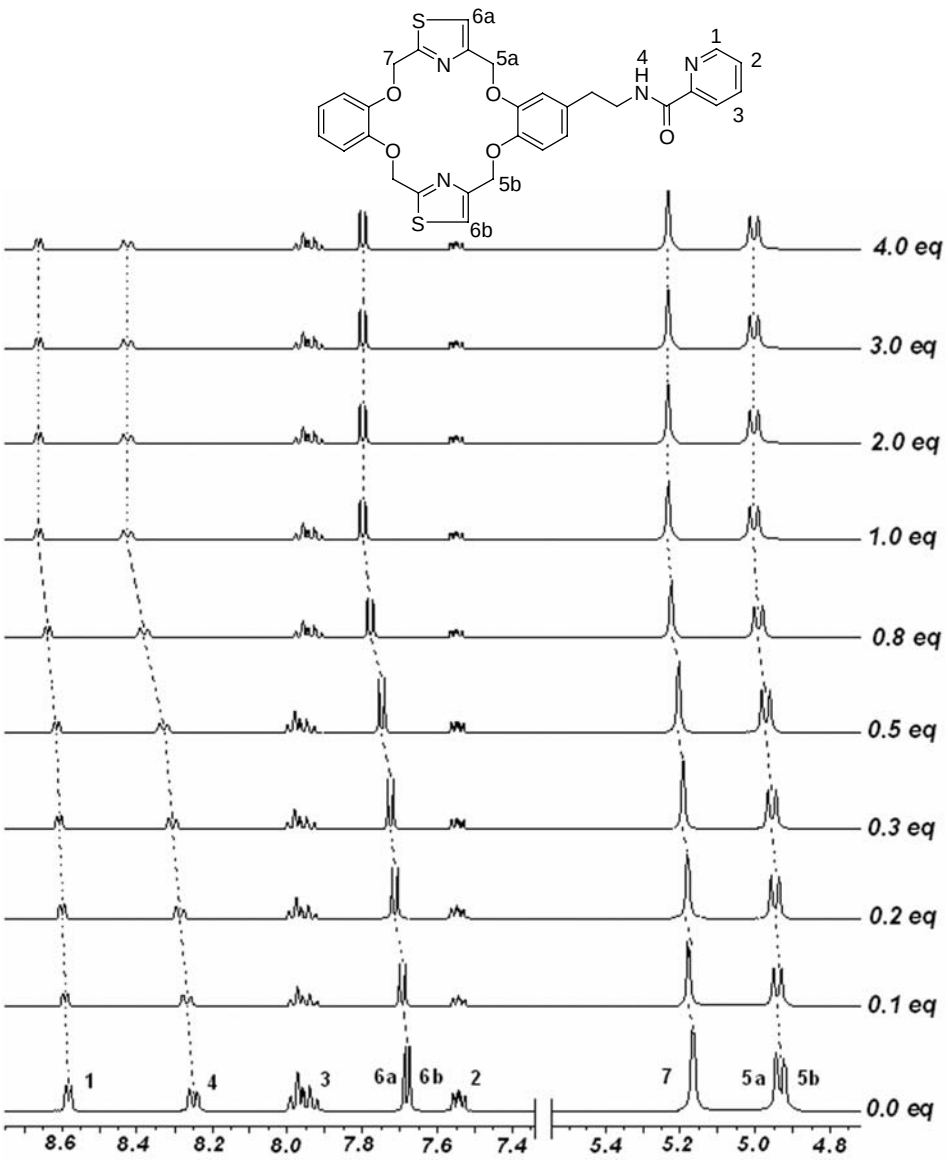

Figure 2. Partial ^{1}H NMR (400 MHz) titration spectra of **4** (4.5×10^{-3} M) with GABA in D_2O–$DMSO_{d6}$ (3:7) at 25°C.

The proton signals of the crown ether moiety also exhibited significant complexation-induced shifts. The methylene protons and the thiazolyl-*H* peaks showed downfield shifts from 4.93 to 5.03 ppm and from 7.68 to 7.80 ppm, respectively, due to the electrostatic interaction between the ether oxygen and thiazole ring nitrogen with NH_4^+ upon addition of 1 equiv. of GABA. These results indicated that **4** acts as a convergent heteroditopic receptor for GABA through hydrogen bonding. From the ^{1}H NMR titration using the nonlinear curve-fitting program WinEQNMR (*28*), the association constant (K_a) of **4** with GABA was calculated to be 3190 M^{-1}. A Job plot of **4** with GABA indicated that **4** formed a 1:1 complex with GABA (Figure 3).

In order to gain insight into the characteristics of the interactions between GABA and **4**, molecular modelling studies were conducted using SPARTAN 04 (Figure 4). The energy-minimised diagram shows that the ammonium ion forms hydrogen bonds disposed in a three-fold symmetry pattern with the thiazole nitrogen ($a = 2.49$ and $b = 2.37$ Å) and the ether oxygen ($c = 1.90$, $d = 2.80$, $e = 2.94$ and $f = 2.55$ Å) (*29*).

The carboxylate anion, on the other hand, formed hydrogen bonds with the amide N-*H* ($g = 1.90$ and $h = 3.08$ Å) and the acidic pyridine-H_1 ($i = 3.52$ Å). Moreover, a conformational change occurred as a result of complex formation; the dihedral angles C_{benzo}-*O*-C_{met}-C_{met} changed from 121° to 110°, thus making the

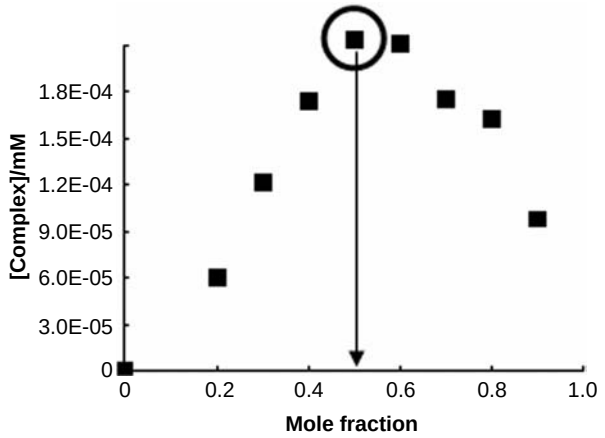

Figure 3. A Job plot of **4** with GABA in D_2O–$DMSO_{d6}$ (3:7) at 25°C.

ammonium ion of GABA the ideal guest in the 18-crown-6 cavity (~1.42 Å). Thus, the optimised structure of the **4**–GABA complex suggests that the design of the new heteroditopic receptor system is capable of the simultaneous coordination of both anionic and cationic groups of the guest species. In order to establish the thermodynamic characterisation of the complexation, isothermal titration calorimetry (ITC) experiments of the receptors with GABA and other amino acids were carried out in DMSO:H_2O (7:3) at 25°C. ITC significantly showed one association constant throughout the titration of **4** with GABA (Figure 5). However, the association constant

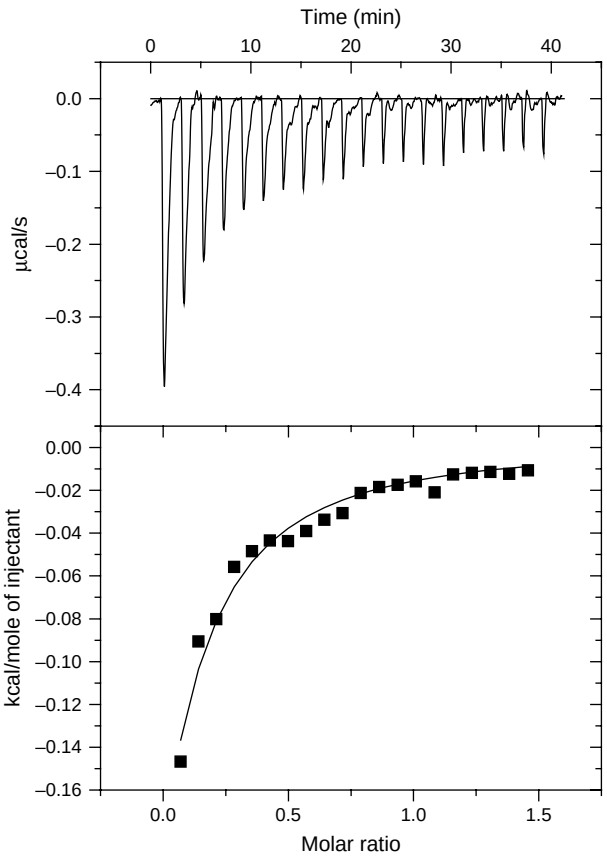

Figure 5. Isothermal calorimetric titration of **4** (1 mM) with GABA (20 mM) in H_2O–DMSO (3:7) at 25°C.

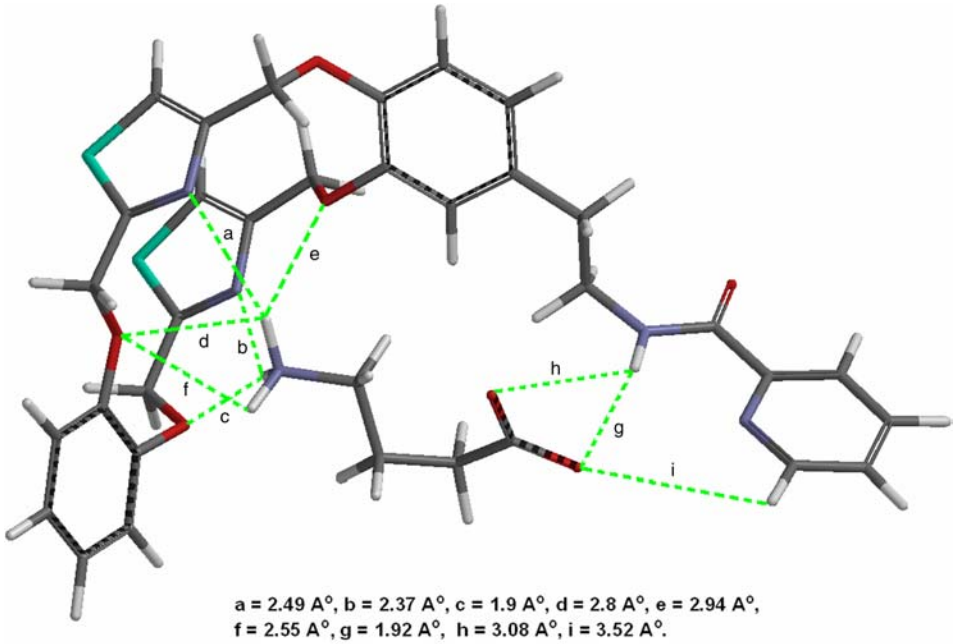

Figure 4. Energy minimised structure of the **4**–GABA complex as predicted by Hartree–Fock 3-21G calculations.

Table 1. Association constants (M^{-1}) and thermodynamic values from ITC for the complexation of amino acids with receptors.[a]

Receptor	Guest	K_a	ΔH	ΔS
4	Gly	115	-2.28×10^3	8.47
	L-Alanine	158	-6.04×10^3	−10.2
	Glutamine	440	-5.64×10^3	−7.17
	Arginine	82	-2.94×10^3	−0.96
	β-Alanine	39	-4.10×10^3	−6.68
	Gly–Gly	1300	-1.61×10^4	−290
	GABA	2220	-1.31×10^5	−418
	5-APA[b]	1400	-6.10×10^4	−187
	6-AHA[c]	1250	-2.09×10^4	−674
5	GABA	19	-1.32×10^3	−15.2
6	GABA	291	-2.05×10^4	−22.2

[a] Determined in H_2O–DMSO (3:7) at 25°C, $[H]_o = 1.0 \times 10^{-3}$ M, $[G]_o = 2.0 \times 10^{-2}$ M.
[b] 5-Aminopentanoic acid.
[c] 6-Aminohexanoic acid.

obtained for **4** with GABA at 50°C was four times less than that observed at 25°C. This might be due to the improper orientation of GABA towards the proper binding site at high temperature. These results suggested that the addition of GABA forms a 1:1 complex with **4** regardless of how much the concentration of GABA is increased.

A similar kind of binding mode was obtained for all other amino acids. Moreover, the complexation of all the amino acids with the receptors was enthalpy stabilised (since all ΔH values are negative; Table 1). The enthalpy change ΔH was obtained directly from the calorimetric experiments, whereas the entropy change ΔS was calculated according to the formula: $\Delta G^\circ = -RT \ln K = \Delta H - T\Delta S$. Other thermodynamic parameters derived from the calorimetric measurements are summarised in Table 1. The association constant of **4** with GABA obtained from the ITC data was 2220 M^{-1}. The control compounds **5** and **6** with GABA had a lower association constant (Table 1). Thus, the covalently connected heteroditopic receptor **4** is a superior GABA receptor capable of binding a carboxylate and an ammonium ion simultaneously in a cooperative fashion through the interaction with hydrogen bonding.

Importantly, the geometry of ditopic receptors must be optimised so that the anion and cation binding sites are located within appropriate proximity to one another to enhance this interaction; incorrect orientation could lead to the ion pair associating outside of the receptor, or with the solvent. That is why the terminal NH_2-to-COOH distance of the amino acids is necessary for their proper fixation onto the receptor molecule, so that the carboxylate group–amide N*H* and pyridine-H_1 and ammonium group–crown cavity interactions can occur simultaneously. Thus, the better the fixation of the amino acid onto the receptor molecule, the higher the stability of the resulting 1:1 adduct. From Table 1, glycine (Gly), L-alanine, phenylalanine and β-alanine due to their short-chain length and arginine, glutamine and Gly–Gly with a longer-chain length had lower association constants with **4** compared with GABA. In the case of receptors **5** and **6**, the absence of the acidic pyridine-H_1 and amide N*H*, respectively, weakened the formation of the hydrogen bond between the carboxylate anion of GABA and the receptors, consequently lowering the association constants. A decrease in the association constant of **4** with 5-APA and 6-AHA compared with GABA indicated that the proper orientations of the ammonium and carboxylate anion are necessary to ideally poise the binding site of the receptor.

3. Experimental section

3.1 General methods

General experimental procedures for melting points, FT-IR spectra, mass spectra and elemental analyses have been described previously (*23b*). ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 Spectrometer with TMS as an internal standard. ^1H NMR titrations were run at 45 mM concentrations, with addition of a 0.25 M amino acid solution. ITC measurements were performed using an Omega titration microcalorimeter. A 15 mM solution of amino acid in 40 times (5 μL injection) was added to a 1 mM receptor solution (1.8 mL) in a calorimetric cell. Flash column chromatography was performed with Merck silica gel 60 (70–230 mesh). All reactions were carried out under an atmosphere of argon. The solution was washed with brines and dried over anhydrous sodium sulphate. 1,3-Dichloroacetone, trifluoroacetic acid, picolinic acid, benzoic acid, 2-(bromomethyl)pyridine, DIC, DCC and HOBT hydrate were purchased from Aldrich Co. and used as received. 1,2-Bis(thioamidomethyloxy)benzene and *N*-Boc-dopamine were prepared with a procedure described in the literature (*23*, *26*).

*3.1.1 Synthesis of 1,2-bis[2(4-chloromethyl)thiazolyl]-methyloxybenzene (**1**)*

A mixture of 1,2-bis(thioamidomethyloxy)benzene (1.00 g, 3.90 mmol) and 1,3-dichloroacetone (1.00 g, 8.58 mmol) in benzene (70 mL) was refluxed for 20 h with the Dean–Stark column to remove water. After the solvent was removed, it was extracted with dichloromethane, dried and concentrated. The residue was purified by column chromatography (elution with EtOAc–hexane 1:4) to yield 1.32 g of **1** (84%). TLC R_f 0.48 (EtOAc–hexane 1:1); m.p. 116°C (CH_2Cl_2–hexane); ^1H NMR ($CDCl_3$) δ 7.30 (s, 2H, Thz-*H*), 6.97 (m, 4H, Ph), 5.39 (s, 4H, OCH_2THz), 4.75 (s, 4H, CH_2Cl); ^{13}C NMR ($CDCl_3$) δ 168.7, 152.3, 148.4, 123.2, 118.7, 115.9, 69.1, 41.1; MS (relative intensity) *m/z* 401 (M^+, 2), 292 (13), 254 (18), 146 (100%); Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_2S_2$: C, 47.88; H, 3.52; N, 6.98; S, 15.98. Found: C, 47.98; H, 3.59; N, 6.94; S, 16.25.

3.1.2 Synthesis of TBC-ethylamine-t-Boc ***(2)***

A mixture of *N-Boc*-dopamine (1.00 g, 3.95 mmol), K_2CO_3 (1.20 g, 8.69 mmol) and NaI (1.30 g, 8.69 mmol) in butanone (100 mL) was heated at 60°C for 1 h, and then a solution of **1** (1.74 g, 4.33 mmol) in butanone (10 mL) was added to the resulting mixture and heated for 6 h. After the solvent was removed, it was extracted with dichloromethane, washed, dried and concentrated. The residue was purified by chromatography (EtOAc–hexane 1:1) to yield 1.65 g of **2** (72%). TLC R_f 0.43 (EtOAc–hexane 2:1); m.p. 164–166°C (CH_2Cl_2–hexane); IR (KBr) 3430, 2346, 2257, 2129, 1649, 1050, 1026. 1002 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.79 (s, 2H, Thz*H*), 7.29–7.16 (m, 2H, Ph), 7.04–6.97 (m, 4H, Ph), 6.76 (m, 1H, Ph), 5.26 (s, 4H, OCH_2Thz), 5.05 (s, 2H, ThzCH_2O), 5.02 (s, 2H, ThzCH_2-O), 3.17 (s, 2H, PhCH_2CH_2NHCO), 2.67 (t, $J = 7.5$ Hz, 2H, PhCH_2CH_2NHCO), 1.38 (s, 9H, -OC(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) δ 163.9, 163.8, 155.9, 152.6, 152.5, 148.1, 148.0, 146.7, 132.6, 122.1, 121.1, 120.9, 120.8, 114.6, 114.0, 113.4, 77.8, 66.1, 65.8, 35.4, 28.7; MS (relative intensity, %) *m/z* 581 (M^+, 14), 189 (100); Anal. Calcd for $C_{29}H_{33}N_3O_6S_2$: C, 59.67; H, 5.70; N, 7.20; S, 10.99. Found: C, 59.78; H, 5.44; N, 7.13; S, 11.07.

3.1.3 Synthesis of TBC-ethylamine ***(3)***

A solution of **2** (1.00 g, 1.72 mmol) in dichloromethane (50 mL) was reacted with trifluoroacetic acid (2.91 g, 25.80 mmol) at room temperature for 12 h. The mixture was neutralised with 1 N sodium hydroxide solution, extracted with dichloromethane, washed, dried and crystallised in a freezer to yield 580 mg of **3** as white solid (70%). TLC R_f 0.13 (CH_2Cl_2–MeOH–NH_4OH 16:5:1); m.p. 171–173°C (CH_2Cl_2); IR (KBr) 3426, 3074, 2930, 1687, 1507, 1246, 1201, 1121, 1022, 799; ^{1}H NMR (DMSO-d_6) δ 7.92 (bs, 2H, $CH_2CH_2NH_2$), 7.84 (s, 1H, Thz-*H*), 7.83 (s, 1H, Thz-*H*), 7.19–7.16 (m, 2H, Ph), 7.08–6.98 (m, 2H, Ph), 7.05 (d, $J = 8.1$ Hz, Ph), 7.01 (bs, 1H, Ph), 6.78 (d, $J = 8.1$ Hz, 1H, Ph), 5.28 (s, 2H, OCH_2-Thz), 5.27 (s, 2H, OCH_2Thz), 5.07 (s, 2H, ThzCH_2O), 5.03 (s, 2H, ThzCH_2O), 3.07 (t, $J = 8.4$ Hz, 2H, $CH_2CH_2NH_2$), 2.82 (t, $J = 8.4$ Hz, 2H, $CH_2CH_2NH_2$); ^{13}C NMR ($CDCl_3$) δ 164.0, 151.9, 147.7, 147.5, 146.6, 129.9, 121.5, 120.7, 113.8, 65.5 (OCH_2Thz), 65.1 (ThzCH_2O), 40.6 (CH_2-CH_2NH_2), 32.6 ($CH_2CH_2NH_2$); Anal. Calcd for $C_{24}H_{23}N_3O_4S_2$: C, 59.86; H, 4.81; N, 8.73; S, 13.32. Found: C, 59.91; H, 4.60; N, 8.88; S, 13.75.

3.1.4 Synthesis of TBC-picolinic acid conjugate (TBC-PAC, ***4****)*

A mixture of picolinic acid (115 mg, 0.92 mmol), DIC (0.15 mL, 0.92 mmol) and HOBT hydrate (135 mg, 0.92 mmol) in dichloromethane (15 mL) was stirred at room temperature for 30 min. A solution of **3** (200 mg, 0.41 mmol) in dichloromethane (5 mL) was added to the resulting mixture, and stirred for 12 h. The mixture was extracted with dichloromethane, washed, dried and concentrated to dryness. The residue was purified by flash chromatography (elution with EtOAc 100%) to yield 158 mg of **4** (64%). TLC R_f 0.10 (100% EtOAc); m.p. 165–167°C (CH_2Cl_2–hexane); IR (KBr) 3446, 2924, 2358, 2235, 2119, 1654, 1508, 1244, 1030, 1009, 825; ^{1}H NMR ($CDCl_3$) δ 8.52 (d, $J = 8.0$ Hz, 1H, Py), 8.29 (bd, $J = 4.5$ Hz, 1H, N*H*CO), 8.04(d, $J = 7.5$ Hz, 1H, Py), 8.00 (dd, $J = 7.5$, 1.5 Hz, 1H, Py), 7.72 (s, 1H, Thz-*H*), 7.71 (s, 1H, Thz-*H*), 7.60 (td, $J = 7.0$, 5.5 Hz, 1H, Py), 7.20 (dd, $J = 5.5$, 5.0 Hz, 2H, Ar), 7.06 (m, 2H, Ar), 7.04 (s, 1H, Ar), 6.87 (d, $J = 8.0$ Hz, 1H, Ar), 5.24 (s, 4H, OCH_2Thz), 5.02 (s, 2H, ThzCH_2O), 4.99 (s, 2H, ThzCH_2O), 3.63 (t, $J = 7.0$ Hz, 2H, CH_2CH_2NH), 2.88 (t, $J = 7.0$ Hz, 2H, CH_2CH_2NH); ^{13}C NMR ($CDCl_3$) δ 166.2, 166.0, 164.3, 152.9, 152.6, 149.9, 149.8, 149.5, 149.4, 148.4, 148.1, 138.4, 133.9, 126.7, 123.8, 123.8, 122.9, 122.9, 118.5, 118.2, 118.1, 118.0, 117.5, 69.4, 69.3, 68.8, 68.4, 41.2, 35.7; Anal. Calcd for $C_{30}H_{26}N_4O_5S_2$: C, 61.42; H, 4.47; N, 9.55; S, 10.93. Found: C, 61.31; H, 4.73; N, 9.30; S, 10.52.

3.1.5 Synthesis of TBC-benzoic acid conjugate (TBC-BAC, ***5****)*

A mixture of benzoic acid (100 mg, 0.82 mmol), DIC (0.13 mL, 1.89 mmol), HOBT hydrate (190 mg, 1.4 mmol) and diisopropylethylamine (1.48 mL, 8.07 mmol) in dichloromethane (50 mL) was stirred at room temperature for 30 min. A solution of **3** (410 mg, 0.80 mmol) in dichloromethane (5 mL) was added to the resulting mixture, and refluxed for 12 h. The mixture was extracted with dichloromethane, washed with brine and dried. The residue was purified by flash chromatography (elution with EtOAc 100%) to yield 374 mg of **5** (78%). TLC R_f 0.40 (100% EtOAc); m.p. 117–118°C (CH_2Cl_2–hexane); IR (KBr) 3430, 2255, 2128, 1652, 1050, 1026, 1004 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.69 (s, 1H, Thz-*H*), 7.67 (s, 1H, Thz-*H*), 7.37 (m, 2H, Ph), 7.30 (m, 2H, Ar), 7.22 (d, $J = 6.0$ Hz, 2H), 7–7.30 (m, 3H), 7.22 (d, $J = 6.0$ Hz, 1H), 6.99 (m, 2H, Ar), 6.94 (d, $J = 8.0$ Hz, 1H), 6.87 (s, 1H, Ar), 6.79 (d, $J = 8.0$ Hz, 1H, Ar), 6.40 (bs, 1H, N*H*CO), 5.23 (s, 2H, OCH_2Thz), 5.23 (s, 2H, OCH_2Thz), 5.02 (s, 2H, ThzCH_2O), 5.00 (s, 2H, ThzCH_2O), 3.64 (t, $J = 6.5$ Hz, 2H, PhCH_2CH_2), 2.84 (t, $J = 6.5$ Hz, 2H, PhCH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 167.9, 167.3, 167.1, 161.6, 151.7, 151.6, 149.8, 149.3, 134.9, 134.4, 131.8, 128.9, 127.3, 124.2, 124.1, 123.4, 119.0, 118.8, 118.3, 118.2, 117.3, 69.1, 68.3, 67.8, 41.6, 35.5; Anal. Calcd for $C_{31}H_{27}N_3O_5S_2$: C, 63.57; H, 4.65; N, 7.11; S, 10.95. Found: C, 63.37; H, 4.86; N, 7.10; S, 11.26.

*3.1.6 Synthesis of TBC-2-pyridylmethyl conjugate (TBC-PMC, **6**)*

A mixture of **3** (200 mg, 0.42 mmol), Et_3N (0.3 mL, 2.1 mmol) and 2-(bromomethyl)pyridine hydrobromide (160 mg, 0.63 mmol) in dry dichloromethane (20 mL) was stirred at room temperature for 10 h. The mixture was extracted with dichloromethane, washed and dried. The residue was purified by flash chromatography (elution with EtOAc 100%) to yield 146 mg of **6** (61%). TLC R_f 0.05 (100% EtOAc); m.p. 128–130°C (CH_2Cl_2–hexane); IR (KBr) 3430, 2255, 2128, 1652, 1050, 1026, 1004 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.64 (d, $J = 5.0$ Hz, 1H, Py), 7.90 (ddd, $J = 8.0, 7.5, 1.6$ Hz, 1H, Py), 7.78 (d, $J = 8.0$ Hz, 1H, Py), 7.40 (m, 1H, Py), 7.26 (s, 1H, Thz-*H*), 7.23 (s, 1H, Thz-*H*), 6.88 (m, 2H, Ar), 6.80 (d, $J = 7.5$ Hz, 1H, Ar) 6.72 (m, 3H), 6.50 (s, 1H), 5.35 (bs, 1H, N*H*CO), 5.13 (s, 4H, OCH_2Thz), 5.03 (s, 2H, ThzCH_2O), 5.02 (s, 2H, ThzCH_2O), 4.04 (m, 2H, NHCH_2Py), 3.29 (m, 2H, PhCH_2CH_2), 2.67 (t, $J = 7.0$ Hz, 2H, PhCH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 169.6, 169.3, 157.8, 157.6, 156.2, 150.0, 149.8, 148.7, 147.0, 146.9, 136.2, 132.0, 124.2, 122.2, 122.0, 120.9, 115.3, 115.3, 115.0, 114.8, 113.0, 112.8, 70.2, 70.0, 48.2, 36.5; Anal. Calcd for $C_{30}H_{28}N_4O_4S_2$: C, 62.92; H, 4.93; N, 9.78; S, 11.20. Found: C, 62.57; H, 4.81; N, 9.80; S, 11.56.

4. Conclusion

We have reported that a new heteroditopic receptor, compound **4**, recognises zwitterionic amino acids such as GABA in polar protic solvents. Compound **4** shows selectivity towards GABA compared with similar amino acids based on the spacer length and orientation. The receptor uses thiazolobenzocrown ether for ammonium binding and amide N*H* with a pyridine-H_1 for carboxylate anion binding simultaneously in a cooperative fashion.

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