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A Series of Ditopic Receptors for Succinic Acid Binding

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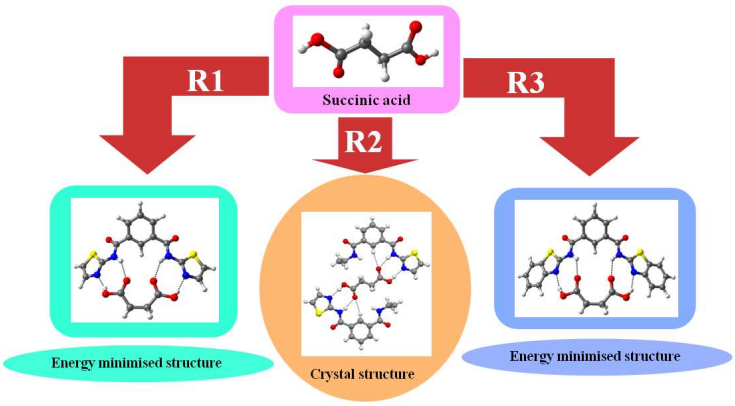
Three *ditopic-abiotic* receptors (**R1, R2** and **R3**) have been designed and synthesised. The receptors have been applied for the recognition of dicarboxylic acids *viz.* malonic, succinic, glutaric and adipic acids. Among them, succinic acid shows the highest binding efficiency to all receptors.



Novelty statement: Recognition of succinic acid is very much important due to their various adverse effects on human health upon prolonged exposure.

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ARTICLE TYPE

A Series of Ditopic Receptors for Succinic Acid Binding †

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Three *ditopic-abiotic* receptors (**R1, R2** and **R3**) have been designed and synthesised in one step. The receptors have been applied for the recognition of dicarboxylic acids *viz.* malonic succinic, glutaric and adipic acids. Among them, succinic acid shows the highest efficiency to all receptors. To support this observation, we have gone through UV-vis, NMR, single crystal X-ray techniques, theoretical calculation and molecular modelling study.

Introduction

The molecular recognition and supramolecular architecture are built up by different hydrogen bonding and nonbonding weak interactions in mimicking assorted phenomena in nature.[1] Heterocyclic synthons in supramolecular assembly produce hydrogen bonding, which has further developed a network by different directional weak interactions.[2] Binding is the most essential factor for the existence and reactivity of the compounds. The expansion of artificial receptors, designed for the detection of biologically important substrates like carboxylic acids and carboxylate anion is an important topic in molecular recognition research.[3] Carboxylate in carboxy-peptidase enzyme is an essential component of numerous metabolic processes like citric acid and glyoxalate cycles.[4] Such type of receptors have received extensive attention in crystal engineering,[5] supramolecular chemistry,[6] molecular biology,[7] and pharmaceutical science.[8] The carboxylic acid has conquered as one of the major targeted guests in which some eminent scientists really did lot of work in the field of molecular recognition with host-guest binding.[9] Tremendous features of carboxylic acid have diverse applications in supramolecular chemistry. Being a useful hydrogen bonding synthon, carboxylic acid has been significantly exploited in generating solid-state supramolecular structures with varied designed or ready-made receptors.[10]

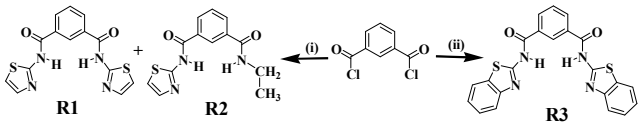
A complete study for discrimination of aromatic dicarboxylic acids over aliphatic dicarboxylic acids and ethylene-linked mixed pyridine diamide receptor have been reported by Goswami et al.[11] In most of the cases, a common strategy was resorted to design receptors that involved linking two aminopyridine groups through the spacer for dicarboxylic acids and certain molecules of biological importance. Azacrown and tetrazole receptors have also potential binding capabilities of carboxylic acids and carboxylate ion as well as their congener acids accordingly.[12] For the last couple of decades, a lot of research works has been published with synthetic fluorogenic chemosensor for dicarboxylic, citric acid, α-keto acid and hydroxyl monocarboxylic acids.[13] The Photoinduced Electron Transfer (PET) sensors, chemosensors were observed to possess binding sites, where recognition of anions like dicarboxylates, pyrophosphate and heavy metal ions have been reported.[14] A thiourea-based receptor has been accounted for selective recognition of fumaric and maleic acids in aqueous medium by Das *et al.* The new fluorescent receptor was applied to explain FRET response on the association of receptor-carboxylic acids.[15]

A principal theme in the design of artificial receptors has been used for rigid molecular components to grip hydrogen-bonding groups at a fixed distance apart. Recently, we have published the recognition studies of a furfurylamine appended ditopic receptor for succinic acid in details.[16] In this current manuscript, we want to report three truncated receptors (**R1**, **R2** and **R3**), which could be effective hosts on the basis of the complementary for succinic acid. The binding components of the receptors are connected through a common isophthaloyl spacer and a separation of the two hydrogen-bonding regions would specify the chain length selectivity of the guest (succinic acid). Due to the widespread use of the acid as ingredients in various foods and their possible adverse influences on human health upon prolonged exposure, it is important to develop an efficient reagent for their recognition and quantitative estimation for the dicarboxylic acid.

Synthesis of receptor 1, 2 and 3

In this context, we have synthesized three receptors by employing isophthaloyl dichloride as a spacer and attaching 2-aminothiazole, 2-aminobenzthiazole and ethylamine groups with it to form pendent receptors **1, 2** and **3**. Mechanistically, all these reaction schemes have one step process and they are afforded in presence of dry Et_3N in CH_2Cl_2 under N_2 atmosphere at room temperature, with a good yield. The receptor **1** (**R1**) is a dipodal receptor, containing two *2*-aminothizole moieties. It has been synthesised by the reaction between isophthaloyl dichloride and 2-aminothiazole compounds in presence of dry Et_3N in dry CH_2Cl_2. From this reaction, we also get receptor **2** (**R2**), in which one 2-aminothiazole and one ethylamine moieties are attached. The ethylamine part breaks from triethylamine, which was used as a base and this part was attached to one acid chloride site of isophthaloyl dichloride to form **R2**. The similar procedure was followed to afford receptor **3** (**R3**). The synthesis of all dipodal receptors **R1, R2** and **R3** are shown in scheme **1**.

Reaction scheme 1



Reagents and conditions: (i) 2-aminothiazole, excess dry Et_3N, dry CH_2Cl_2, 12 hrs, rt, (ii) 2-aminobenzthiazole, dry Et_3N, dry CH_2Cl_2, 12 hrs, rt.

Results and discussion

All the receptors have been characterised by FTIR, NMR, and Mass spectroscopic techniques. We have also used single crystal X-ray only for **R2-succinic acid** complex along with common analytical techniques described before.

Complexation and binding studies between R1 and dicarboxylic acids by UV-vis method

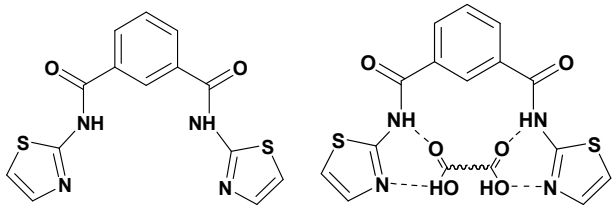
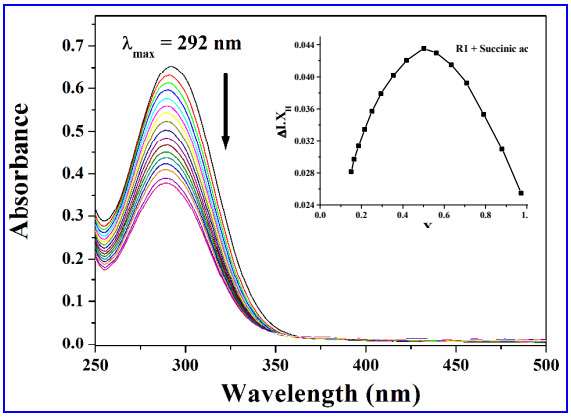


Fig. 1: R1 and probable mode of complexation with dicarboxylic acid

UV-vis titration has been carried out taking a stock solution of **R1** with all four dicarboxylic acids stated above. A stock solution has been prepared by dissolving 0.11 mg of **R1** in 25 ml chloroform (concentration = 1.33 x 10^{-5} M) and exhibited absorbance maxima at λ_{max} = 292 nm in chloroform (**Fig. 2a**). The guest solutions were also set up using 2% DMSO in $CHCl_3$ to make it homogeneous. The continuous decrease of absorbance until 1:1 complexation between host-guest upon addition of guest solution was also observed. This incident was easily explained through the titration curve i.e. after 1:1 complexation the curve is quite parallel to *X*-axis, which implies no more shifting of absorbance after 1:1 complexation (**Fig. 2b**). The association constant and the stoichiometry of complexation were calculated from the titration experiments using previously mentioned method used for **R1** and dicarboxylic acids.



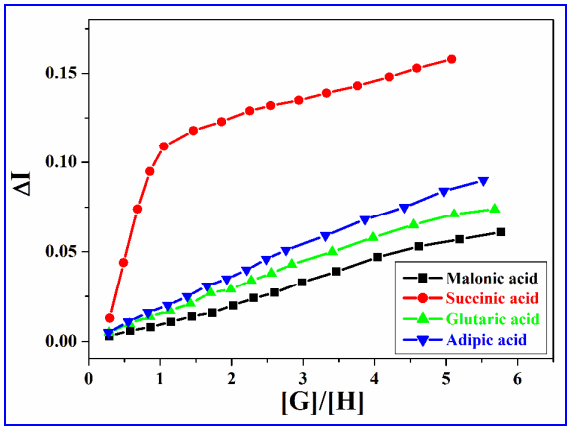


Fig. 2: (a) Titration spectra of **R1** with succinic acid (inset: Job's plot), **(b)** titration curve of **R1** with all four acids in 1% DMSO-$CHCl_3$ using UV-vis method.

The reciprocal of concentration of guest (1/[G]) and corresponding reciprocal of absorbance difference (1/ΔI) could be conveniently plotted for the calculation of association constant (K_a) using linear method and the graph has been put in the Electronic Supplementary Information (ESI).[17] The values of K_a are tabulated in **Table 1,** which shows the highest association constant value of **R1** with succinic acid. Therefore it can be concluded that **R1** is the optimised receptor only for succinic acid compare to other three acids. The space between two binding points of **R1** is only fitted with the length of succinic acid. So, the K_a value is much higher (3.48 x 10^4 M^{-1}) for succinic acid than glutaric acid and other acids.

Table 1: Calculation of the Association Constant (K_a) of **R1** and corresponding ΔG values by UV-vis techniques using linear method.

Guest	UV-vis method (K_a, M^{-1})	$\Delta G_{UV\text{-}Vis}$, (Kcal.M^{-1}) (25^0C)
Malonic acid	0.19 (±0.01) x 10^4	-4.47
Succinic acid	**3.48 (±0.01) x 10^4**	**-6.19**
Glutaric acid	0.56 (±0.01) x 10^4	-5.11
Adipic acid	0.13 (±0.01) x 10^4	-4.24

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Complexation study by NMR method between R1 and succinic acid

As the association constant (K_a) calculated from UV-vis technique was higher for **R1-succinic acid** complex rather than malonic, glutaric and adipic acids, we only carried out ^{1}H-NMR titration experiment for **R1-succinic acid** complex. In case of **R1** itself, the distinguish *amide* and *peri* protons appear at δ 11.65 ppm and δ 8.82 ppm in $CDCl_3$. A remarkable downfield shifting (δ 11.65 ppm to 12.06 ppm, Δδ = 0.41 ppm) of the amide protons has occurred, when **R1** formed a complex with succinic acid in 2% d_6-DMSO-$CDCl_3$. No significant change of chemical shift values (δ 8.82 ppm to 8.88 ppm, Δδ = 0.06 ppm) has been taken place for *peri* proton of **R1** under complexation. Other protons also have not shown any noticeable shifting upon complexation in NMR (**Fig. 3**).

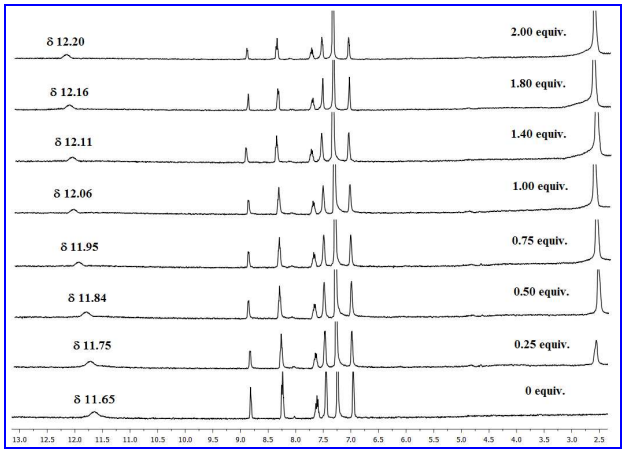

Fig. 3: ^{1}H-NMR titration spectra of **R1** with succinic acid in 2% d_6-DMSO-$CDCl_3$.

From this titration, we can find out the proportion of the complex i.e. the ratio of host (**R1**) and guest (succinic acid) and the association constant (K_a). It has been found that after 1:1 complexation the downfield shifting of amide protons decreases, which was also shown graphically by plotting [G]/[H] vs Δδ and X_H vs X_H.Δδ (**Fig. 4a**). We get maxima at XH = 0.5 in Jobs plot (**Fig. 4b**), which implies that 0.5 mole fraction of host binds 0.5 mole fraction of guest i.e. 1:1 ratio. The association constant (K_a) of **R1-succinic acid** has been found 0.17×10^3 M^{-1} and the curve is placed in the Appendix.

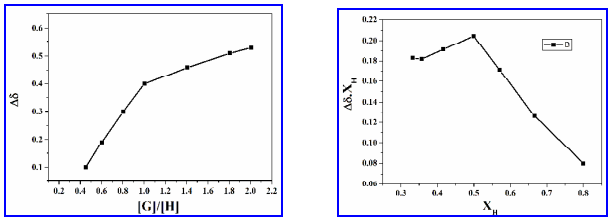

Fig. 4: (a) ^{1}H-NMR titration curve, (b) Jobs plot of **R1** with succinic acid in 2% d_6-DMSO-$CDCl_3$.

Theoretical calculation and molecular modeling studies of R1 and dicarboxylic acids

For theoretical calculation and molecular modelling, all the complex structures including the receptors (**R1, R2 and R3**) were optimized in Semi-empirical AM1 method. All calculations have been performed with Gaussian03 program package with the aid of the Gauss-View and Chemcraft visualization program.

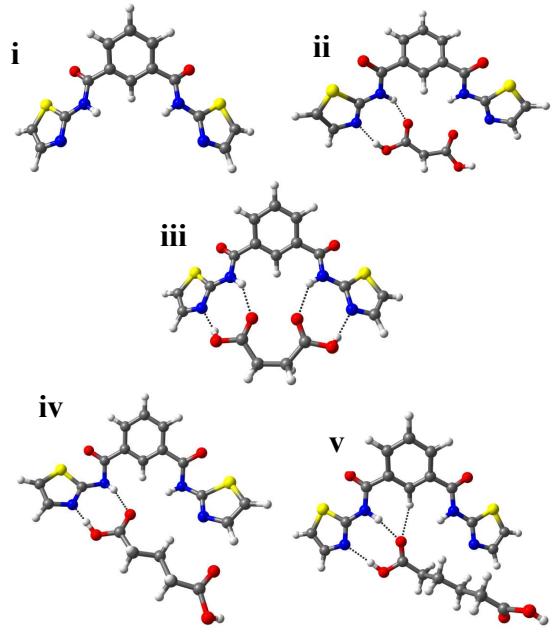

Fig. 5: Molecular modelling of (i) **R1**, (ii) **R1**-malonic, (iii) **R1**-succinic, (iv) **R1**-glutaric and (v) **R1**-adipic acids with their hydrogen bonding interactions, accordingly.

From the above semi-empirical energy minimised structures, it is clear that the **R1** bind succinic acid most effectively as **R1** forms four hydrogen bonding interactions with succinic acid. Here, both the carboxylic acid groups take part in interactions with the receptor's N-H and thiazole ring 'N' (**Fig. 5**). This fact is also supported by the calculated semi-empirical energy values (**Table 2**), which shows that **R1-Succinic acid** complex having the lowest energy is most stable among all the complex structures.

Table 2: Calculated semi-empirical energies of **R1** and its complexes

Complexes of R1 with dicarboxylic acids	Semi empirical energy (a.u.)
Receptor 1 (R1)	0.0893
Malonic acid complex	-0.2314
Succinic acid complex	**-0.2436**
Glutaric acid complex	-0.2378
Adipic acid complex	-0.2381

Complexation study by UV-vis method between R2 and dicarboxylic acids

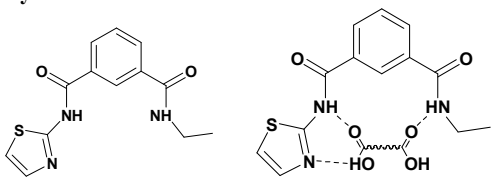

Fig. 6: R2 and probable mode of complexation with dicarboxylic acid.

Similar type of observation was also obtained during the titration

of **R2** with mentioned dicarboxylic acids. Purified solid **R2** (0.11 mg in 25.0 ml, concentration = 1.59 x 10^{-5} M) was dissolved in UV-grade $CHCl_3$ into a volumetric flask. From the stock solution, we have taken 2.0 ml for UV-vis titration. The peak maximum has appeared at λ_{max} 288 nm and the absorbance was regularly lower down upon guest addition (**Fig. 7a**). After certain time, the absorbance does not change longer, this makes clear that 1:1 complexation is formed between host and guest in the solution. This phenomenon can be explained using Jobs plot and titration curve (**Fig. 7a inset**). When we have plotted a graph X_H *vs* ΔI. X_H, a peak maxima has been found at $X_H = 0.5$ in Jobs plot, which implied that one equivalent amount of host (**R2**) was saturated by one equivalent guest molecule (**Succinic acid**). The same result could be explained from the stoichiometry curve (**Fig. 7b**).

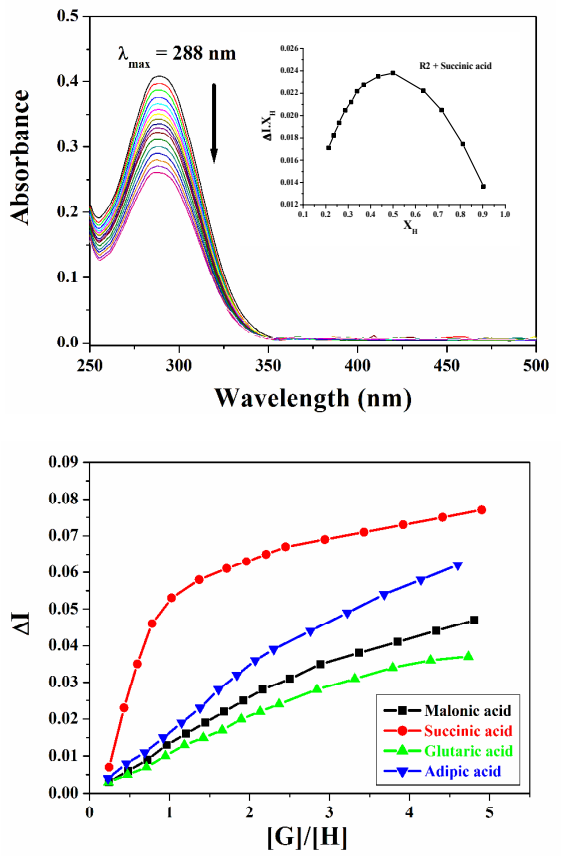

Fig. 7: (a) Titration spectra, inset: Job's plot of **R2** with succinic acid, **(b)** titration curve of **R2** with all four acids in 1% DMSO-$CHCl_3$ using UV-vis method.

To calculate association constant (K_a) using linear regression analysis, we plotted 1/[G] *vs* 1/[ΔI] and we found straight line for each case. The association constant calculation graph has been placed in ESI and the values are provided in **Table 3**. According to the association constant values, succinic acid has the better binding affinity (K_a = 3.33 x 10^4 M^{-1}) towards **R2**, which clearly indicates that the cavity size of **R2** is quite fit to allow for entering succinic acid and get complexed.

Table 3: Calculation of the Association Constant (K_a) of **R2** and corresponding ΔG values by UV-vis techniques using linear method.

Guest	UV-vis method (K_a, M^{-1})	$\Delta G_{UV\text{-}Vis}$, (Kcal.M^{-1}) (25^0C)
Malonic acid	0.19 (±0.01) x 10^4	-4.47
Succinic acid	**3.33 (±0.01) x 10^4**	**-6.17**
Glutaric acid	0.39 (±0.01) x 10^4	-4.90
Adipic acid	0.10 (±0.01) x 10^4	-4.09

Complexation study by NMR method between R2 and dicarboxylic acids

To confirm the structure of **R2**, we have gone through ^{1}H-NMR of pure compound in $CDCl_3$. Eventually, it has been observed that the amide protons attached to thiazole moiety and ethyl group of **R2** appeared at same position, δ 11.36 ppm. Though, it is quite unusual that both protons should not come at same place. Comparing integral values of ^{1}H NMR spectrum, it becomes clear that the peak at 11.36 ppm contained two protons which have been vanished after shaking with one drop of D_2O. The *peri* protons of **R2** became visible at δ 8.62 ppm, which changed its position slightly upon complexation. The NMR titration spectra have been given in **Fig. 8**, which shows that the amide peak has been shifted to δ 11.91 ppm (Δδ 0.55 ppm) upon addition of 0.9 equivalent succinic acid and after addition of 1.0 equivalent guest the peak was too broad to detect its accurate position and it was merged with the base line of NMR spectra (**Fig. 8**).

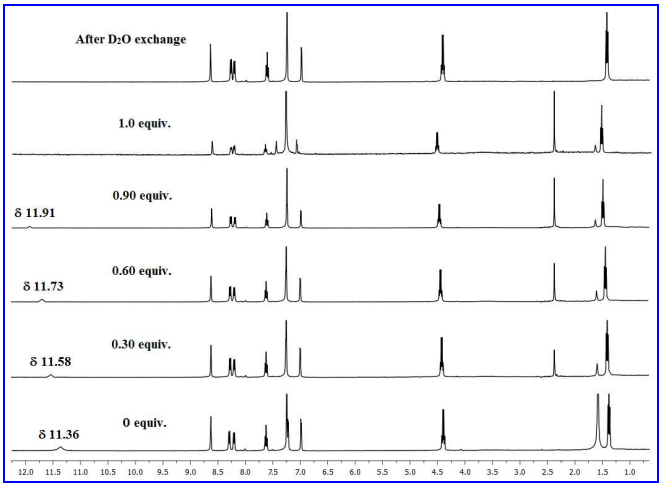

Fig. 8: ^{1}H-NMR titration spectra of **R2** with succinic acid in 2% d_6-DMSO-$CDCl_3$.

As it was described that the distinguish amide peak was vanished upon addition of 1.0 equivalent dicarboxylic acid, it is impossible to determine stoichiometry as well as association constant (K_a) using NMR technique.

Single crystal X-ray analysis of R2-succinic acid complex

The supramolecular structures were also observed by increasing the chain of the dicarboxylic acid beyond the optimum. By increasing the length of dicarboxylic acid, the formation of ribbon or dynamic helical supramolecular structure was also obtained.[10b]

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We find here a complex structure of **R2-succinic acid** based on hydrogen bonding, which is quite dissimilar with the dynamic supramolecular structure.[18] The above mentioned complex crystal has been originated as 2:1 complex of *N1-ethyl-N3-(thiazol-2-yl)isophthalamide* (**R2**) and succinic acid with a triclinic fashion in *P-1* space group (CCDC No. 1012219). Two molecules of each synthon of the complex formed a unit cell (**Fig. 9**). But only one molecule of **R2** and one molecule of succinic acid are present in an asymmetric unit. The X-ray structure of the **R2-succinic acid complex** shows that the amide proton [N2-H2C] forms a hydrogen bond with O3-atom of carboxylic acid moiety, i.e. [N2-H2C…..O3] bond has been found. Another hydrogen bonding interaction was observed through OH [O4-H4C] center of carboxylic moiety with thiazole ring N [N3], i.e. [O4-H4C.....N3]. Third hydrogen bonding interaction was found between donor 'O' atom [O3] of carboxylic acid moiety and *peri* proton [C9-H9] of **R2**. All the hydrogen bonding distances and angles are given in **Table 4**. No such interaction was observed for N1-H1 aliphatic amide proton, i.e. where aliphatic amine (ethylamine) attached to isophthaloyl dichloride. 1:1 complex interacts with another complex through hydrogen bonding in opposite arrangement and finally makes a 2:1 complex.

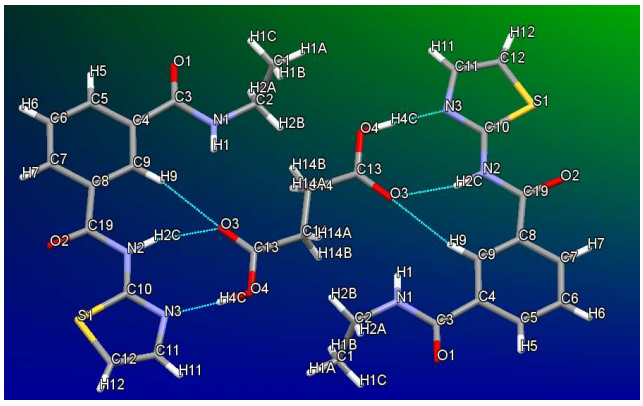

Fig. 9: (a) X-ray structure of **R2** viewed through crystallographic *a*-axis (gray for C, off white for H, red for O and blue for N, blue lines represent H-bond).

Table 4: Hydrogen bond distances (Å) and angles (°) for **R2-Succinic acid complex** structure (D, donor; H, hydrogen; A, acceptor).

D–H ⋯ A	D–H	D⋯A	H⋯A	< D–H⋯A
N2-H2C ⋯ O3	0.89(3)	2.825(2)	1.96(3)	163(3)
O4-H4C ⋯ N3	0.94(5)	2.625(3)	1.70(5)	167(4)
C9-H9 ⋯ O3	0.93	3.344(3)	2.45	162.5

Theoretical calculation and molecular modelling studies of R2 and dicarboxylic acids

Theoretically calculated energy minimised structures represent the binding of succinic acid with **R2** in a very effective way through five hydrogen bonding interactions (**Fig. 10**). It has been found that receptor's cavity is optimized for succinic acid and both the carboxylic acid ends of succinic acid take part in hydrogen bonding interaction with **R2** showing lowest stablisation energy [-0.3763 a.u., **Fig. 10(iii)**]. Other complex structures of **R2** do not exhibit good compatible modes of binding with a shorter chain length of malonic acid and longer chain length of glutaric and adipic acids.

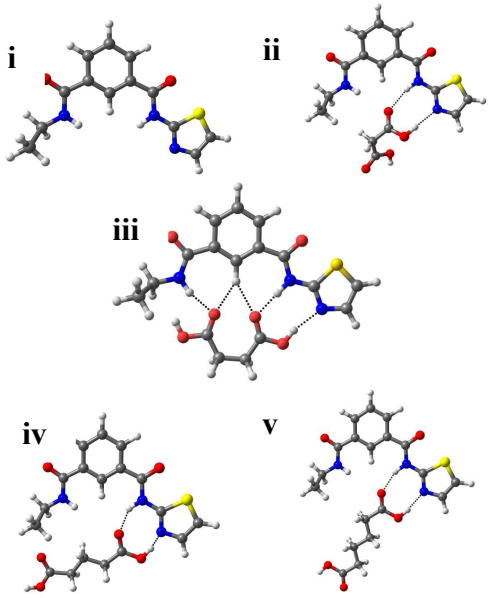

Fig. 10: Molecular modelling of (i) **R2**, (ii) **R2**-malonic, (iii) **R2**-succinic, (iv) **R2**-glutaric and (v) **R2**-adipic acids with their hydrogen bonding interactions, accordingly.

The calculated semi-empirical energy values indicate that **R2-Succinic acid** complex has the lowest energy (**Table 5**), that means most stable among all the complex structures.

Table 5: Calculated semi-empirical energies of **R2** and its complexes

Complexes of R2 with dicarboxylic acids	Semi empirical energy (a.u.)
Receptor **2** (**R2**)	-0.0011
Malonic acid complex	-0.3176
Succinic acid complex	**-0.3763**
Glutaric acid complex	-0.3257
Adipic acid complex	-0.3274

Complexation study by UV-vis method between R3 and dicarboxylic acids:

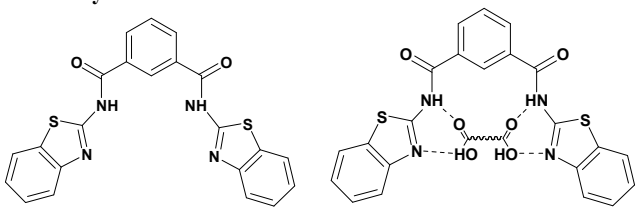

Fig. 11: R3 and probable mode of complexation with dicarboxylic acid

R3 (0.18 mg, concentration = 1.67 x 10^{-5} M) was dissolved in $CHCl_3$ (25.0 ml) for photophysical studies. From this, 2.0 ml of host solution was taken to perform titration experiment. In electronic spectrum, a peak maximum has been found at λ_{max} 307

nm and the absorbance was going to decrease regularly upon addition of guest solution (**Fig. 12a**). After 1:1 complexation, the absorbance no longer changed, i.e. host became saturated by guest molecules. This observation has been explained by Jobs plot and titration curve also (**Fig. 12b**).

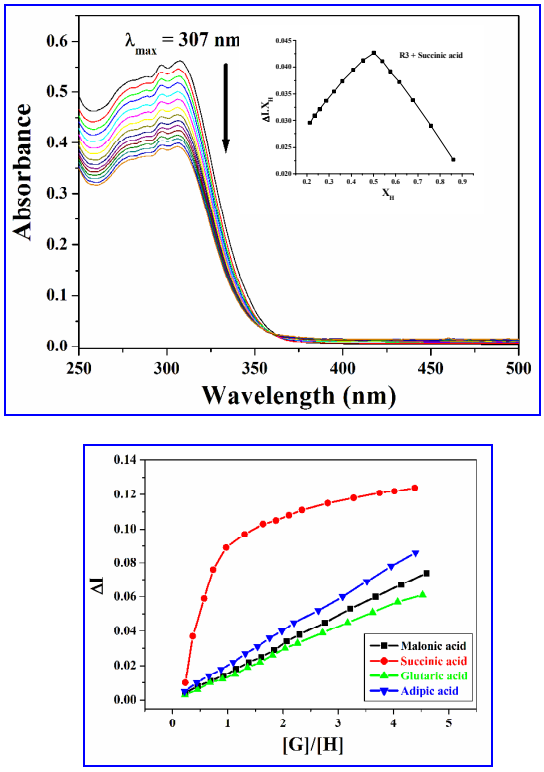

Fig. 12: **(a)** Titration spectra, inset: Job's plot of **R3** with succinic acid, **(b)** titration curve of **R3** with all four acids in 1% DMSO-$CHCl_3$ using UV-vis method.

Complexation study of R3 with dicarboxylic acids by fluorescence method:

R1 and **R2** are not fluorescent enough for complexation studies but **R3** exhibit good fluorescent properties due to the presence of two benzothiazole moieties. So the complexation study of **R3** with different dicarboxylic acids has been carried out by fluorescence spectroscopic analysis in $CHCl_3$. When it was excited at λ_{max} 307 nm, an emission spectrum with peak maxima at 438 nm has been observed. Significant enhancing of fluorescence intensity has been observed with the gradual addition of succinic acid (0 to 3.0 equivalents) to the solution of **R3** (c = 1.67 x 10^{-5} M) [**Fig. 13**]. For comparative study, analogous investigations were carried out, where slight enhancing has been occurred on gradual addition of the other dicarboxylic acids. The limit of detection (LOD) calculation using standard method[19] shows that **R3** can sense succinic acid up to very low concentration (3.21 × 10^{-7} M) (see ESI).

During fluorescence titration experiment, the receptor **R3** forms complex with succinic acid as initial absorption intensity of **R3** changes sharply and then very slowly. When we have plotted concentration of guest [G] upon concentration of host [H] against change in absorbance (ΔI), i.e. ([G]/[H]) vs ΔI, it has been observed that the curve after [G]/[H] = 1 tends to be parallel to X-axis and a break point of slope of the titration curve implies 1:1 complexation between **R3** and succinic acid, (**Fig. 13**). No such break point has been found for other acids, because of their very weak interactions with **R3.**

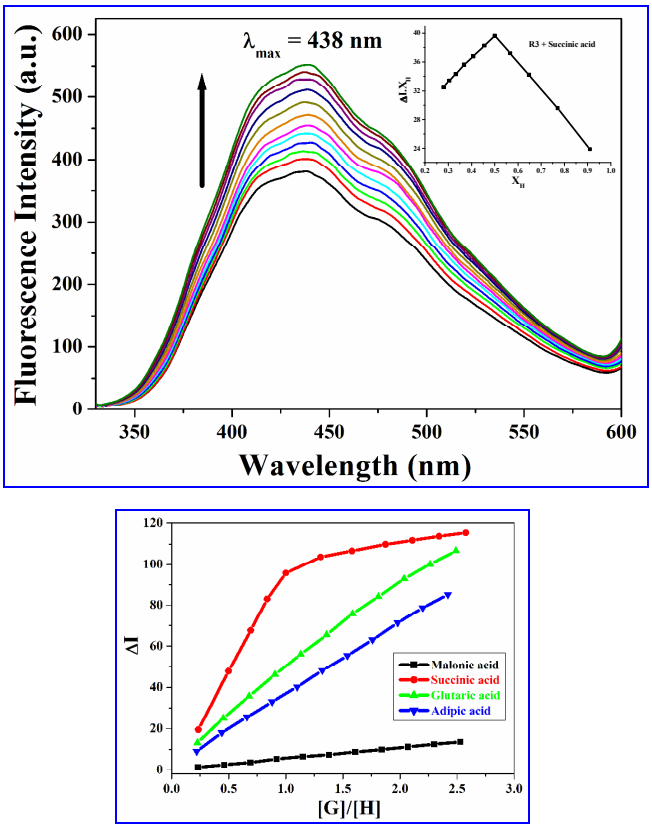

Fig. 13: **(a)** Fluorescence titration spectra, inset: Job's plot of **R3** with succinic acid, **(b)** titration curve of **R3**, measured at 438 nm with all four acids in 1% DMSO-$CHCl_3$.

The association constants for **R3** with all four dicarboxylic acids in UV-Vis and fluorescence techniques have been determined using linear regression analysis (see *ESI*) and the same procedure has been followed which was previously mentioned. The values were mentioned in the **Table 6** which shows that succinic acid has the highest binding constant compare to malonic, glutaric and adipic acids. As the cavity size concern, **R3** is optimized to the chain length selectivity of only succinic acid.

Table 6: Calculation of the Association Constant (K_a) of **R3** and corresponding ΔG values by UV-vis techniques using linear method.

Guest	UV-vis method (K_a, M^{-1})	Fluorescence method (K_a, M^{-1})
Malonic acid	0.13 (±0.01) x 10^4	0.14 (±0.01) x 10^4
Succinic acid	**3.26 (±0.01) x 10^4**	**5.29 (±0.01) x 10^4**
Glutaric acid	0.48 (±0.01) x 10^4	0.30 (±0.01) x 10^4
Adipic acid	0.11 (±0.01) x 10^4	0.28 (±0.01) x 10^4

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Complexation study by NMR method between R3 and dicarboxylic acids

The peak at δ 8.16 ppm was revealed for two amide protons attached to benzothiazole and *peri* protons here appeared at δ 8.59 ppm i.e. more downfield to amide protons in ^{1}H NMR spectra. After addition of 1.0 equivalent of succinic acid, the peak of amide protons have been downfield shifted to δ 8.75 ppm (δΔ 0.59 ppm) and no such change of δ values has been noted on excess addition. The detail NMR titration spectra of **R3** *vs* **succinic acid** has been placed in **Fig 14**.

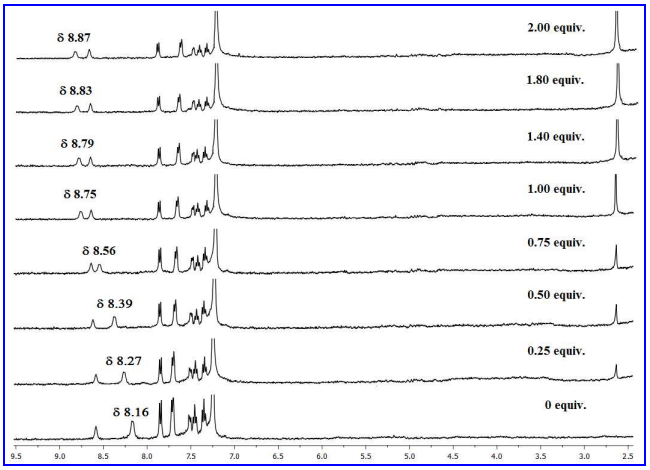

Fig. 14: ^{1}H-NMR titration spectra of **R3** with succinic acid in 2% d_6-DMSO-$CDCl_3$.

The ratio of complexation between **R3** and succinic acid has been calculated from NMR titration experiment. When we draw a curve X_H *vs* $\Delta\delta.X_H$ (Jobs plot), a peak maxima has been found at 0.5 which implies 1:1 complexation of **R3-succinic acid** (**Fig. 15a**). The same observation has been again explained from titration curve ([G]/[H] *vs* Δδ) as the curve is quite parallel to *X*-axis after 1:1 complexation [**Fig. 15b**]. The association constant (K_a) of **R3**-succinic acid has been found 0.18×10^3 M^{-1} and the curve is placed in the Appendix.

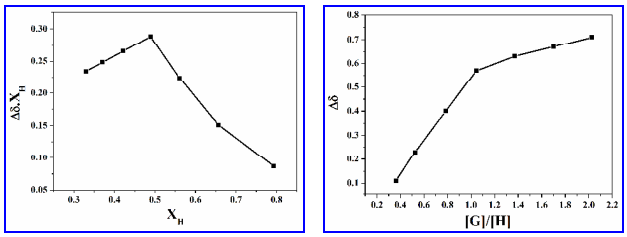

Fig. 15: (a) Job's plot **(b)** titration curve of **R3** with succinic acid in $CDCl_3$ in NMR.

Theoretical calculation and molecular modelling studies of R3 and dicarboxylic acids

The following semi-empirical energy minimised structures exhibit that **R3** bind succinic acid most effectively as **R3** forms four hydrogen bonding interactions with succinic acid. Here, both the carboxylic acid groups take part in interactions with the receptor's N-H and benzothiazole ring 'N' (**Fig. 16**). This fact is also supported by the calculated semiemperical energy values (**Table 7**), which shows that **R3-Succinic acid** complex is the most stable among all the complex structures.

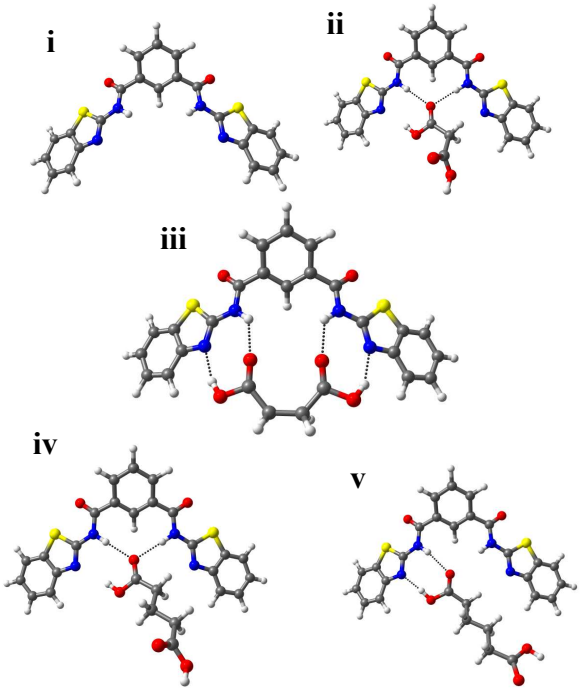

Fig. 16: Molecular modelling of (i) **R3**, (ii) **R3**-malonic, (iii) **R3**-succinic, (iv) **R3**-glutaric and (v) **R3**-adipic acid with their hydrogen bonding interactions accordingly.

Table 7: Calculated semi-empirical energies of **R3** and its complexes.

Complexes of R3 with dicarboxylic acids	Semi empirical energy (a.u.)
Receptor **3** (**R3**)	0.1403
Malonic acid complex	-0.1783
Succinic acid complex	**-0.1915**
Glutaric acid complex	-0.1857
Adipic acid complex	-0.1862

Conclusions

In conclusion, we report here the design and synthesis of three different isophthalamide receptors (**R1, R2** and **R3**) and the binding properties have also been studied with four different dicarboxylic acids using NMR and UV-Vis spectroscopic techniques. In addition, a series of titration experiments were carried out with dicarboxylic acids, and the resulting association constants (K_a) were presented, where the highest value corresponds to succinic acid complex. The theoretical calculation using *Gaussian03* program suggested that the complex of these receptors with succinic acid have the highest number of hydrogen bonding interactions in comparison to other complexes. Also the semi empirical energy of all the receptor-succinic acid complexes have lowest value that means they are most stable than other

dicarboxylic acid complexes. Single crystal X-ray analysis has confirmed the structures of **R2-succinic acid** complex where the probable binding modes of **R2** with succinic acid are clearly identified in solid state. We have also carried out the complexation study of these receptors with succinic acid using ^{1}H NMR spectroscopic technique where the downfield shifting of the NH and *peri* protons unambiguously concluded the same complexation phenomena. Thus we can conclude that all these receptors are adequate design for the recognition of succinic acid, whose length and steric features correspond to the interior cavity of the receptors.

Experimental section

X-ray crystallography

The data of **R2-succinic acid** complex was collected on a Bruker AXS SMART APEX II diffractometer equipped with area detector system, using Mo Kα radiation with graphite monochromatization (λ = 0.71073 Å) at T = 293 (2) K. The structures were solved by direct methods SHELXS-97 (Sheldrick, 1990) and refined by full-matrix least-squares methods; using SHELXL-2013 (Sheldrick, 2013).

Crystallographic data (excluding structure factors) for the structure, reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as a supplementary publication no. **CCDC 1012219**. The crystallographic data are summarized in **Table 8**.

Table 8: Crystallographic data of R2-succinic acid complex:

Crystal Data:	**R2-succinic acid** complex
CCDC No.	1012219
Formula:	$C_{15}H_{16}N_3O_4S$
Formula Weight:	334.37
Crystal System:	Triclinic
Space group:	P-1
a, b, c [Å]:	8.085 (3), 9.738 (3), 10.671 (4)
α, β, γ (°):	78.516 (9), 83.189 (9), 68.866 (9)
V [Å]^3:	766.9 (4)
Z:	2
D(calc) [g/cm^3]:	1.448
μ(Mo-Kα) [mm^{-1}]:	0.236
F(000):	350
Crystal Size [mm]:	0.16 x 0.14 x 0.12
Temperature (K):	293(2)
Radiation [Å]: Mo-Kα	0.71073
$\theta_{Min-Max}$ [°]:	1.95, 26.997,
Dataset:	-10≤ h ≤ 10
	-12 ≤ k ≤ 10
	-13 ≤ l ≤13
Tot., Uniq. Data, R(int):	10043, 3232, 0.0229
Observed data [I > 2.0 σ(I)]:	2609
Refinement: N_{ref}, N_{par}:	3232, 216
R, wR2, S:	0.0537, 0.1550, 1.079
Max. & Min. Shift/Error:	0.005, 0.001
Min. & Max. Resd. Dens. [e/ Å^3]:	-0.705, 0.482

General

All solvents were dried prior to use by common methods. Silica gel (60–120 and 100–200 mesh) were used for all chromatographic purifications. The reaction was carried out under nitrogen atmosphere in anhydrous solvents. Melting points (mp) were recorded using a *Remco* hot-coil stage melting point apparatus. NMR spectra were recorded in $CDCl_3$ with TMS as the internal standard in Bruker 400 MHz instruments. Chemical shifts are given in δ (ppm) scale and *J* values in Hertz. IR spectra were measured in KBr disk using *Spectrum 2000 Perkin-Elmer Spectrometer* and UV–vis spectra were recorded on *UV-1800 Shimadzu Spectrophotometer*.

Synthesis of N1, N3-di(thiazol-2-yl)isophthalamide (R1)

Isophthaloyl dichloride (500.0 mg, 2.46 mmol) was taken into dry dichloromethane (10.0 mL) in a double necked round bottomed flask. 2-Aminothiazole (739.9 mg, 7.39 mmol) mixed with triethylamine (2.0 mL) was drop wise added to the acid chloride solution through a time duration of 30 minutes. The total system was fully kept under nitrogen atmosphere. After 12 hours stirring, the reaction was quenched with distilled water. Now, the mixture was extracted with $CHCl_3$ for several times and dried over anhydrous sodium sulphate to afford the crude product. Then pure **R1** was isolated from the crude mixture by column chromatography technique using 1% methanol in $CHCl_3$ as eluent. (Off-white solid, yield: 30 %).

Spectral data of R1

Melting Point: Above 250^{0}C.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 11.65 (bs, 2H), 8.82 (s, 1H), 8.24 (d, 2H, J = 7.2 Hz), 7.61 (t, 1H, J = 7.6 Hz), 7.45 (s, 2H), 6.96 (s, 2H).

TOF-MS ES$^+$ (m/z, %): 352.92 (M-1+23, 80), 330.95 (M, 95), 326.01 (100), 304.03 (75).

FT-IR (KBr, cm^{-1}): 3455, 2929, 1674, 1618, 1548, 1276, 1093, 698.

Synthesis of N1-ethyl-N3-(thiazol-2-yl)isophthalamide (R2)

Isophthaloyl dichloride (500.0 mg, 2.46 mmol) was taken into dry dichloromethane (10.0 mL) in a double necked round bottomed flask. 2-Aminothiazole (493.3 mg, 4.93 mmol) mixed with excess triethylamine (10.0 mL) was drop wise added to the acid chloride solution through a time duration of 30 minutes. The total system was fully kept under nitrogen atmosphere. After 12 hours stirring, the reaction was quenched with distilled water. Now, the mixture was extracted with dichloromethane for several times and dried over anhydrous sodium sulphate to afford the crude product. Then pure **R2** was isolated from the crude mixture by column chromatography technique using $CHCl_3$ as eluent. (White solid, yield: 65 %).

Spectral data of R2

Melting point: Above 250^{0}C.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 11.36 (bs, 2H), 8.63 (s, 1H), 8.29 (d, 1H, J = 8.0 Hz), 8.20 (d, 1H, J = 8.0 Hz), 7.62 (t, 1H, J = 8.0 Hz), 7.22 (d, 1H, J = 3.6 Hz), 6.98 (d, 1H, J = 3.2 Hz), 4.39 (q, 2H, J = 7.2 Hz), 1.38 (t, 3H, J = 7.2 Hz).

TOF-MS ES$^+$ (m/z, %): 298.94 (M+23, 40), 276.95 (M+1, 100).

FT-IR (KBr, cm^{-1}): 3171, 2942, 1718, 1675, 1548, 1304, 1237,

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1090,721.

Synthesis of N1,N3-di(benzo[d]thiazol-2-yl)isophthalamide (R3)

Isophthaloyl dichloride (200.0 mg, 0.98 mmol) was taken into dry dichloromethane (10.0 mL) in a double necked round bottomed flask. 2-Aminobenzothiazole (443.9 mg, 2.95 mmol) mixed with triethylamine (2.0 mL) was drop wise added to the acid chloride solution through a time duration of 30 minutes. The total system was fully kept under nitrogen atmosphere. After 12 hours stirring, the reaction was quenched with distilled water. Now, the mixture was extracted with dichloromethane for several times and dried over anhydrous sodium sulphate to afford the crude product. Then pure **R3** was isolated from the crude mixture by column chromatography technique using 1% methanol in $CHCl_3$ as eluent. (White solid, yield: 70 %).

Spectral data of R3

Melting point: Above 250^0C.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.59 (s, 1H), 8.17 (bs, 2H), 7.85 (d, 2H, J = 7.6 Hz), 7.71 (d, 4H, J = 6.8 Hz), 7.51 (dd, 1H, J = 3.6 Hz), 7.46 (t, 2H, J = 7.6 Hz), 7.35 (t, 2H, J = 7.6 Hz).

TOF-MS ES$^+$ (m/z, %): 431.83 (M+1, 30), 430.82 (M, 100).

FT-IR (KBr, cm^{-1}): 3458, 1689, 1659, 1606, 1559, 1305, 752.

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†Electronic Supplementary Information (ESI) available: ^{1}H-NMR spectra of all receptors and their complexes, association constant calculation curve, LOD calculation, CCDC No. of **R2-succinic acid** complex is **1012219**, See DOI: 10.1039/b000000x.

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