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Original article

# Synthesis and structure guided evaluation of estrogen agonist and antagonist activities of some new tetrazolyl indole derivatives☆

Uma Sharan Singh [a], Ravi Shankar [a], Gaya P Yadav [c], Geetika Kharkwal [b], Anila Dwivedi [b], Govind Keshri [b], M.M. Singh [b], P.R. Moulik [c], K. Hajela [a,*]

[a] *Medicinal and Process Chemistry Division, Central Drug Research Institute, Lucknow 226001, India*
[b] *Endocrinology Division, Central Drug Research Institute, Lucknow 226001, India*
[c] *Molecular and Structural Biology Division, Central Drug Research Institute, Lucknow 226001, India*

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**Abstract**

Several regioisomeric tetrazolyl indole derivatives with structurally modified alkyl substituents at the tetracyclic indole nitrogen containing *N*-ethyl amino tetrazole moiety have been synthesized and screened for their ER binding affinity, agonist (estrogenic), antagonist (antiestrogenic) and anti-implantation activities. *N*-2 regioisomers were found to be moderately antagonists and one compound showed 100% contraceptive efficacy at 10 mg/kg dose. Molecular docking studies carried out in comparison to estradiol and raloxifene showed different binding modes of the two regioisomers to the binding site.


*Keywords:* Tetracyclic; Indoles; Tetrazolyl; Estrogen agonist; Antagonist; Anti-implantation

## 1. Introduction

Despite the remarkable progress in the synthesis of various structurally modified selective estrogen mimetics that can act as antagonists for breast/uterine cancer or which can interfere with the endocrine mediated events leading to implantation of the fertilized ovum to the endometrium (anti-implantation agents) or as progesterone antagonists which could affect early post-implantation events, researchers in this field still face considerable challenges for want of an ideal molecule with superior pharmacology and reduced side effects [1]. As the molecular structure of the hormones and their receptors associated with reproductive processes are being unraveled, the desire for better and safe contraceptive drugs has driven efforts to increase the chemical diversity in non-steroidal molecules to act as estrogen antagonists for fertility control and in diseases like uterine or breast cancer.

Specific molecular recognition of heterocyclic compounds in particular of nitrogen heterocycles has been of considerable importance in biological chemistry [2]. Among the nitrogen heterocycles, the indole nucleus is a fundamental constituent of a large number of natural and synthetic products with biological activities [3]. The 2-phenyl indole nucleus caught the attention of chemists and biologists as a possible estrogen mimetic due to its structural similarity to estradiol. Several estrogen antagonists have been developed incorporating the 2-phenyl indole moiety for example ZK119010 [4] (**1**), pipendoxifene (ERA-923, **2**), bazedoxifene (TSE-424, **3**) [5] and have resulted as promising antiestrogens or selective estrogen receptor modulators (SERMs). Recently, some tetracyclic indoles like indenoindoles (**4**) and benzocarbazoles (**5**) have also been shown to possess mixed estrogen agonist/antagonist activity [6,7] (chart 1).These tetracyclic compounds are more rigid than the 2-phenyl indoles and resemble the natural estrogens in respect of binding affinity to the estrogen receptor.

☆ CDRI Comm. No. 7101.
* Corresponding author. Fax: +91 522 2623405.
*E-mail address:* hajelak@yahoo.com (K. Hajela).

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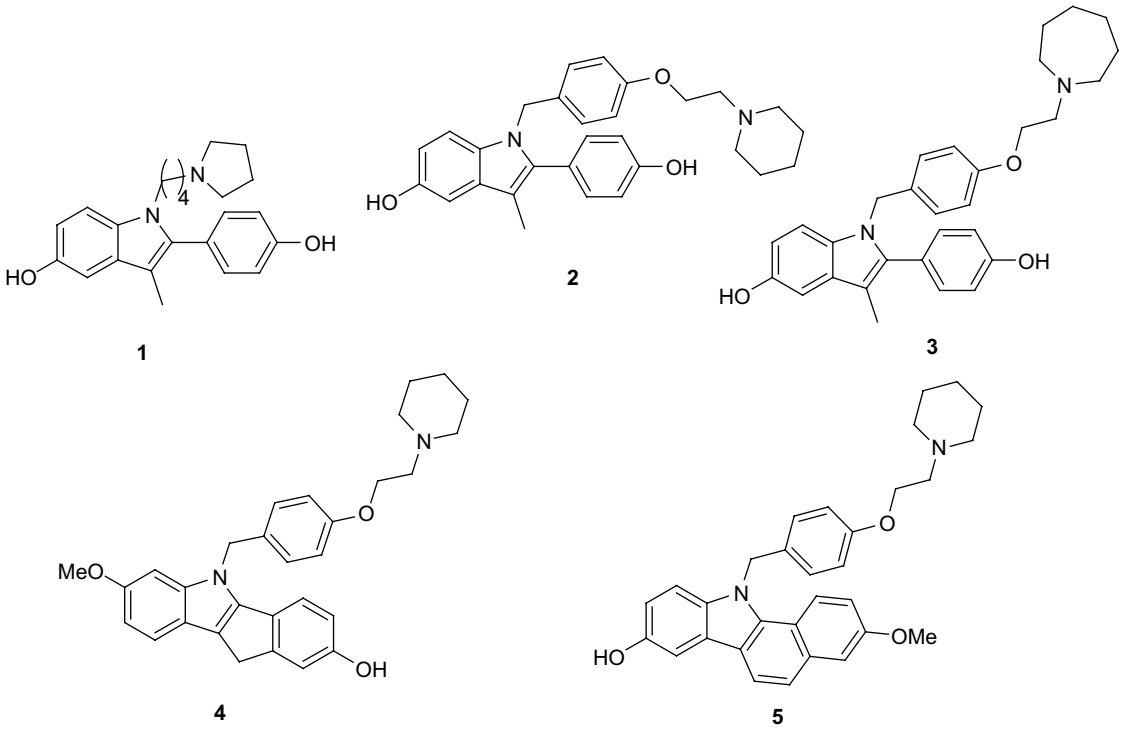



Chart 1. Structures of some important estrogen antagonists (SERMs).

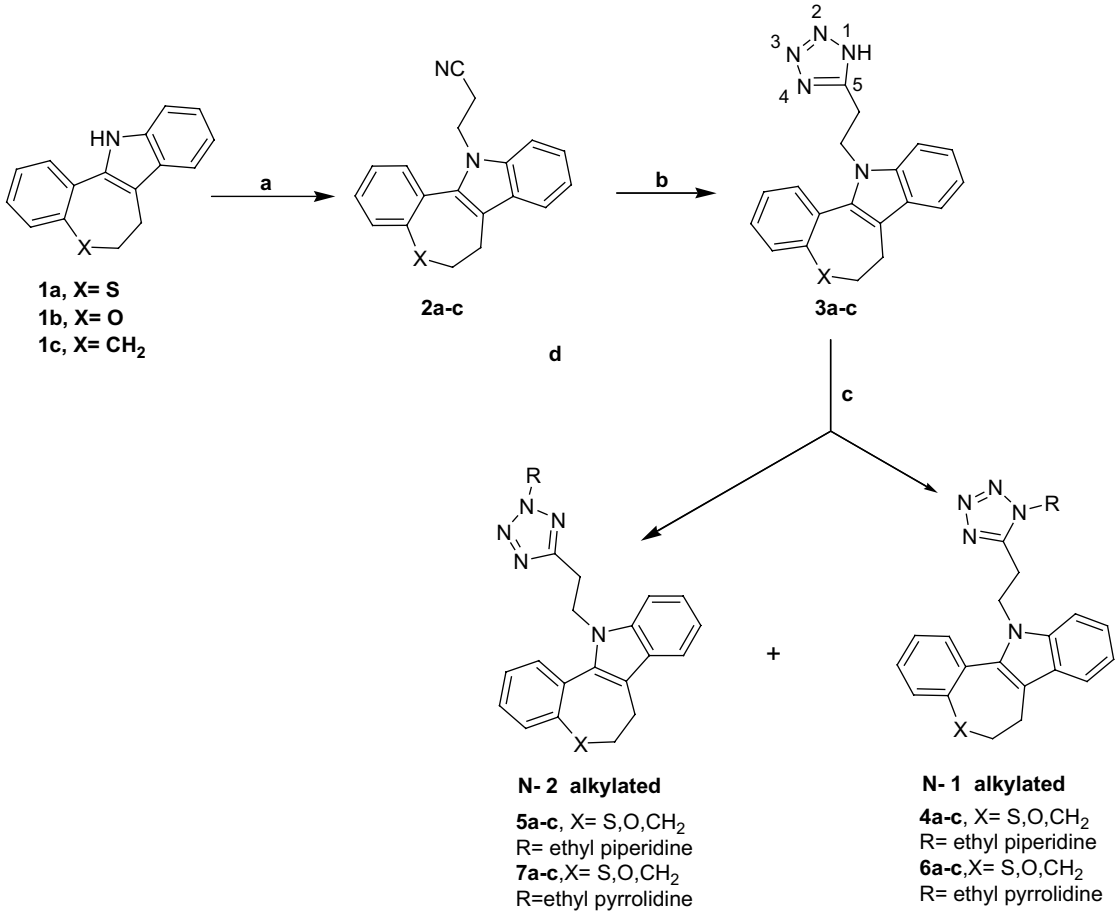
Some tetracyclic indole derivatives synthesized in our group have also shown potent anti-bone resorptive activity [8].

Structural studies on the estrogen receptors (ER) have suggested that there is an ample unoccupied space within the ligand binding domain (LBD) of ER and it can accommodate a wide variety of structurally distinct ligands in its binding pocket [9]. The antagonistic activity of non-steroidal antiestrogens is due to the presence of a long carbon chain with a terminal amino functionality at an appropriate position on the ligand is now well established [10,11]. This side chain, an integral part of almost all non-steroidal antiestrogens establishes hydrophobic interactions with amino acids of the estrogen receptor in the ligand binding domain [12]. Nature of side chain is also an important determinant of selective action and it has been demonstrated that even small changes in the nature or structure of side chain result in large changes in the tissue selectivity profile of the molecule [13]. This realization of the important role of side chain in modulating activity of ER modulators in various physiological processes has resulted in extensive research in this area.

In medicinal chemistry, tetrazole group is often used as metabolism resistant isosteric replacement of acidic groups and also the delocalization of the negative charge around the tetrazole ring is considered favorable for better receptor–substrate interaction [14]. To extend the scope of ER ligands through modification of basic side chain, it was thought to synthesize new molecules with altered carbon chain through insertion of 5-substituted *N*-ethyl amino tetrazolyl moiety in place of conventional phenoxy alkyl amino group at the nitrogen of tetracyclic indole scaffold used as core structure for mimicking 17β-estradiol. The purpose is to facilitate favorable receptor–ligand recognition due to greater distribution of charge around the tetrazole group for enhanced binding affinity/activity and to find new estrogen antagonists with promising anti-implantation (post-coital contraceptive) activity [15,16]. The present paper describes the synthesis of these novel indole derivatives and their biological efficacy.

## 2. Chemistry

The synthesis of the desired tetrazolyl indole derivatives was carried out starting from benzothiepino, benzoxepino and benzocyclohepta indoles **1a–c**. These were synthesized using classical Fisher indole synthesis [17] by reacting seven membered ketones namely, dihydro-2*H*-benzo[*b*] thiepin-5-one, dihydro-2*H*-benzo[*b*]oxepin-5-one and terahydrobenzocyclohepten-5-one with phenyl hydrazine. Cynoethylation of **1a–c** with acrylonitrile in benzyl trimethyl ammonium hydroxide (Triton B) formed the respective *N*-indolyl propionitriles **2a–c** which were cyclised in good yields to 12-[2-(1*H*-tetrazol-5-yl)-ethyl] indole derivatives **3a–c** with sodium azide and ammonium chloride in dry DMF. Tertiary amino alkyl groups were introduced in the tetrazole ring through *N*-alkylation with 1-(2-chloroethyl)pyrrolidine or piperidine hydrochlorides in refluxing dry acetone and triethyl amine. *N*-alkylation of 5-substituted tetrazoles [18] occured on both the tautomeric nitrogen atoms *N*-1 and *N*-2 and in each case both the regioisomers **4a–c** and **5a–c** and **6a–c** and **7a–c** were isolated by purification through column chromatography (Scheme 1).




Scheme 1. Reagents and conditions: (a) acrylonitrile, Triton B, (b) sodium azide, $NH_4Cl$, dry DMF, $\Delta$ (125–130 °C) (c) 1-(2-chloroethyl)pyrrolidine or piperidine hydrochloride, triethyl amine, dry acetone, reflux.

Their structures were confirmed by spectroscopic analysis and X-ray crystallographic studies. In *N*-1-alkylated tetrazoles, the chemical shift of the methylene protons attached to *N*-1 were shifted upfield whereas in *N*-2 alkylated compounds the methylene protons were shifted downfield due to conjugative effect. Compounds **4a** and **5a** were picked up for X-ray analysis to assign the molecular structure for the two regioisomers. Diffraction quality crystals were prepared by slow crystallization from methanol. The structure and molecular conformations are shown in the ORTEP diagram (Fig. 1).

The crystal data of **4a**: $C_{26}H_{30}N_6S$, $M = 458.62$, monoclinic, $P2_{1/c}$, $a = 11.466(2)$ Å, $b = 23.115$, 29.564 (1) Å, $c = 21.518(2)$ Å, $\beta = 94.57(1)°$, $V = 2352.2(5)$ Å$^3$, $Z = 4$, $D_c = 1.295$ g cm$^{-3}$, $\mu$ (Mo K$\alpha$) $= 0.16$ mm$^{-1}$, $F$ (000) $= 976$, rectangular colourless crystal, size $= 0.25 \times 0.2 \times 0.2$ mm, 5441 reflections measured ($R_{int} = 0.0202$), 4147 unique, $wR_{2 =} 0.0948$ for all data, conventional $R = 0.0428$ [$(\Delta/\sigma)_{max} = 000$)] on $F$-values of 2853 reflections with $I > 2\sigma(I)$, $S = 1.016$ for all data and 299 parameters.

The crystal data of **5a**: $C_{26}H_{30}N_6S$, $M = 458.62$, tricyclic, $P$-1, $a = 12.092(2)$ Å, $b = 12.239(2)$ Å, $c = 16.180(2)$ Å, $\alpha = 90.490(10)$, $\beta = 93.140(10)°$, $\gamma = 98.180(10)$, $V = 2366.3(6)$ Å$^3$, $Z = 2$, $D_c = 1.287$ g cm$^{-3}$, $\mu$ (Mo K$\alpha$) $= 0.16$ mm$^{-1}$, $F(000) = 976$, rectangular colourless crystal, size $= 0.25 \times 0.2 \times 0.2$ mm, 9647 reflections measured ($R_{int} = 0.0202$), 8345 unique, $wR_2 = 0.1855$ for all data, conventional $R = 0.0871$ [$(\Delta/\sigma)_{max} = 000$)] on $F$-values of 2589 reflections with $I > 2\sigma(I)$, $S = 0.934$ for all data and 596 parameters.

## 3. Molecular docking studies protocol

Docking, molecular dynamics, energy minimization and molecular graphics works were performed on a silicon graphics octane workstation. The genetic algorithm of AutoDock 3.0 has been employed for docking the tetrazoyl indole derivatives into the active sites of estrogen receptor.

The crystal structure of estrogen receptor LBD in complex with the endogenous estrogen, 17ß-estradiol (II) and raloxifene [19] and reference protein coordinates used for docking were recovered from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/home) (entry codes 1ERE and 1ERR, respectively). Ligand structures of **4a** and **5a** were obtained in pdb-format from its crystal structure. The advanced docking program AutoDock 3.0 was used to perform the automated molecular docking for both compounds. The

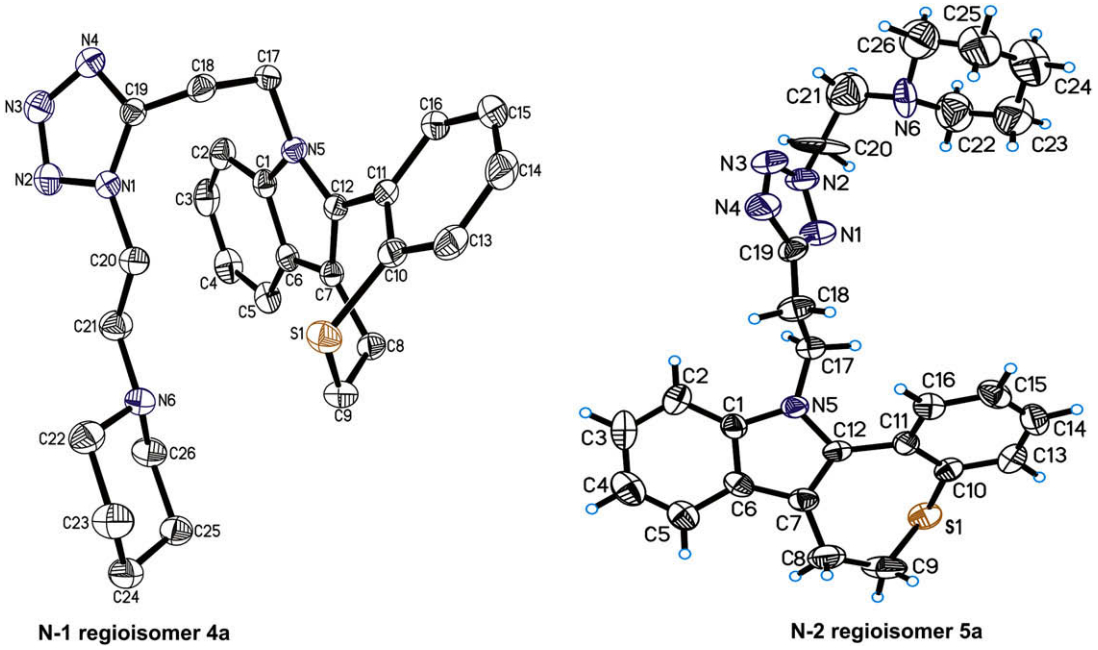



Fig. 1. The *ORTEP* diagram showing the molecular conformations of **4a** and **5a**.

whole docking operation could be stated as follows. First, the ligand molecules were checked for polar hydrogens and assigned for Gasteiger–Huckel [20] partial atomic charges. Flexible torsions were defined with the help of AutoTors. This allowed the conformational search of ligands during the process of docking. The PDBQ file was created for ligands. For macromolecules ER, polar hydrogens were added and Kollman- all-atom [21] atomic charges were taken and the atomic solvation parameters were also assigned using the ADDSOL utility of AutoDock 3.0.5 program. Second, the 3-D grid of 0.375 Å resolutions was centered on the active site using AutoGrid algorithm [22] to evaluate the interaction energies between the ligands and the ER LBD. In this stage, the ER was embedded in the 3-D grid and a probe atom was placed at each grid point. The affinity and electrostatic potential grid were calculated for each type of atom in the ligands. Third, a series of the docking parameters were set on. Not only the atom types but also the generations and the number of runs for GA algorithm were edited and properly assigned. The number of generations, energy evaluations, and GA runs were sets of 27,000, 200,000 and 15, respectively. Finally, the docked complexes of ligand–receptor are selected according to the criteria of interacting energy combined with geometrical matching quality. These complexes were used for comparative study and correlation between the activity and its structural conformations. The total binding free energy was empirically calibrated based on the above-stated terms and set of coefficient factors. The same rational was applied to the system of **4a** and **5a** compounds and estrogen receptor. The total binding free energy and corresponding inhibitory constant between compound and ER was calculated according to the algorithm in the AutoDock 3.0 program [23].

## 4. Results and discussion

### 4.1. Biological results

All the compounds were evaluated for relative ER binding affinity using competitive binding assay, employing radio labeled estradiol ($^3$H-$E_2$) as the reference compound [24]. Briefly, the uterine cytosol (obtained from immature 20–21 days old $E_2$ primed rats) was incubated with $^3$H-estradiol in the absence or presence of various concentrations of test compounds for 22 h at 4 °C. $^3$H-$E_2$ bound and free fractions were separated by charcoal adsorption method. The relative binding affinity of each test compound was calculated from the graph plotted between percent bound radioactivity versus molar concentration of the test substance. At 50% inhibition, log of the competitor concentration relative to that of estradiol expressed the affinity of the test compound to ER relative to estradiol. This when multiplied with 100 gave the % value designated as RBA.

The results of binding studies summarized in Table 1 showed that both regioisomers exhibited weak binding affinity to the estrogen receptor. *N*-2 regioisomers were marginally better in their binding than *N*-1 regioisomers. Lack of free hydroxyl groups at appropriate positions in the tetracyclic indole core may be responsible for the low RBA values.

The screening results of estrogen agonist and antagonist activities showed *N*-1 regioisomers **4a–c** and **6a–c** eliciting substantial agonist activity (uterine weight gain of 23–61%), respectively, whereas *N*-2 alkylated isomers **5a–c** and **7a–c** were less estrogenic. The *N*-2 isomers also showed moderate antagonistic character (Table 1).

To see the effect of moderate antagonism of *N*-2 regioisomers on anti-implantation activity (Table 1) screening

Table 1
Biological activity data of *N*-1 and *N*-2 regioisomers

| Compd. no. | Substituents | | Dose[a] (mg/kg/day) | RBA (% of $E_2$) | Estrogenic activity[b] | | Antiestrogenic activity | | Anti-implantation activity[c] |
|---|---|---|---|---|---|---|---|---|---|
| | X | R | | | Uterine weight mg/kg, body wt. | % Gain | Uterine weight mg/kg, body wt. | % Inhibition | |
| Control | | | | | 17.06 ± 2.11 | | | | |
| EE | | | | | | | 118.10 ± 1.15 | | |
| **4a** | S | Ethyl piperidine | 10 | <0.001 | 20.33 ± 1.23 | 23 | 101.0 ± 4.33 | 2 | NA |
| **4b** | O | Ethyl piperidine | 10 | <0.001 | 23.50 ± 2.20 | 44 | 87.50 ± 4.82 | 1 | NA |
| **4c** | $CH_2$ | Ethyl piperidine | 10 | <0.001 | 20.06 ± 0.93 | 40 | 118.03 ± 1.25 | – | NA |
| **6a** | S | Ethyl pyrrolidine | 10 | <0.001 | 22.66 ± 2.09 | 39 | 90.33 ± 6.75 | – | NA |
| **6b** | O | Ethyl pyrrolidine | 10 | <0.001 | 21.90 ± 3.23 | 40 | 79.03 ± 8.93 | – | NA |
| **6c** | $CH_2$ | Ethyl pyrrolidine | 10 | 0.0125 | 27.43 ± 2.06 | 61 | 110.15 ± 5.26 | 7 | 67% |
| **5a** | S | Ethyl piperidine | 10 | 0.0125 | 16.60 ± 1.25 | – | 85.14 ± 4.25 | 6 | NA |
| **5b** | O | Ethyl piperidine | 10 | 0.011 | 20.0 ± 1.0 | 28 | 93.9 ± 9.45 | – | NA |
| **5c** | $CH_2$ | Ethyl piperidine | 10 | 0.011 | 14.80 ± 0.05 | – | 82.23 ± 4.94 | – | NA |
| **7a** | S | Ethyl pyrrolidine | 10 | <0.001 | 21.40 ± 0.30 | 25 | 116.75 ± 6.05 | 1 | NA |
| **7b** | O | Ethyl pyrrolidine | 10 | 0.04 | 23.90 ± 2.05 | 29 | 104.10 ± 2.68 | 12 | NA |
| **7c** | $CH_2$ | Ethyl pyrrolidine | 10 | 0.16 | 23.63 ± 2.68 | 33 | 103.13 ± 5.12 | 3 | 100% |
| | | | 5 | | | | | | 67% |

[a] Five animals were used for each dose test.
[b] Control group of animals received the vehicle alone. The values represent the mean uterine wt. and activity expressed as % increase over that of control used as basal value.
[c] NA = all rats pregnant.

data showed that majority of *N*-1 and *N*-2 regioisomers were inactive in being devoid of any contraceptive activity and this validated their estrogenic character. Only one *N*-2 regioisomer **7c** showed 100% contraceptive efficacy at 10 mg/kg dose. At a lower dose of 5 mg/kg, it was only 67% effective.

### 4.2. Molecular docking studies

By looking at the spatial conformations of the two regioisomers **4a** and **5a** shown in the crystal structure (Fig. 1), the display of low order of antagonism shown by *N*-2 regioisomers was surprising. It can be clearly seen that the carbon chain in *N*-2 regioisomer **5a** orients itself perpendicular to the indole core structure giving the favorable conformation shown by most of the estrogen antagonists. This molecular similarity was further substantiated by superimposition of *N*-2 regioisomer **5a** over 17β-estradiol and raloxifene as shown in Fig. 2. The relative disposition of the tetracyclic rings of **5a** superimposed closely over both the endogenous hormone and raloxifene and also the side chain is well overlayed on the raloxifene side chain. In contrast, the carbon chain in *N*-1 regioisomer **4a** bends inwards over the tetracyclic indole scaffold giving a folded molecule which may give a better fit into the receptor cavity as shown by pure agonists.

To get an insight of the binding mode of each regioisomer through the interaction of salient amino acids known to anchor both the estrogenic and antiestrogenic ligands in the binding pocket of LBD of ER and to explain the low potency of *in vivo* biological effects, molecular docking studies were performed with both regioisomers **4a** and **5a**. The interaction mechanism currently suggested is that both estrogenic (estradiol) and antiestrogenic (raloxifene) ligands anchor to the binding pocket (aminoacids Glu353 and Arg394) by means of the OH group located at their phenolic site. The second OH group interacts with His524 in both the ligands, whereas the side chain in raloxifene is projected towards Asp351 (Fig. 3(a) and (b).

Though it is suggested that a molecule may not adopt similar conformation as shown by the crystal structure in the LBD domain, it was observed that on docking of crystal structure of **4a**, the molecule adopts a very unfavorable orientation as shown in Fig. 4(a). The tetracyclic core fails to penetrate the ER binding pocket and occupies a space in the cavity by displacing all the salient amino acids responsible for binding of the ligand from the binding pockets. The side chain is placed far away from Asp351 and since the molecule shows no interaction with any of the amino acids, it justifies the weak binding affinity and agonist activity shown by *N*-1 isomers. However, on docking of *N*-2 regioisomer **5a** (Fig. 4(b)), the molecule assumes orientation somewhat

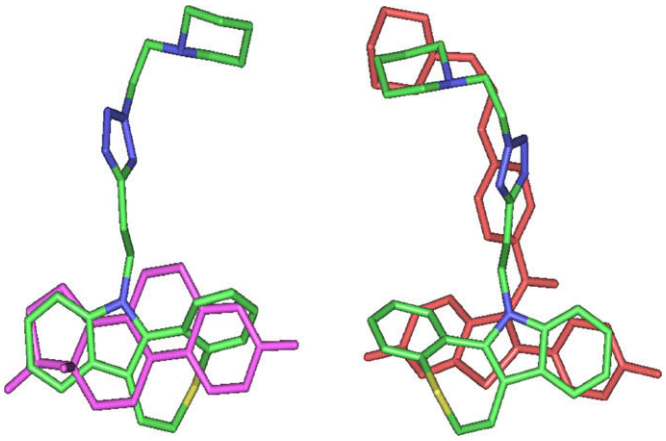


Fig. 2. A flexible overlay of molecule **5a** with estradiol and raloxifene showing significant molecular similarity.

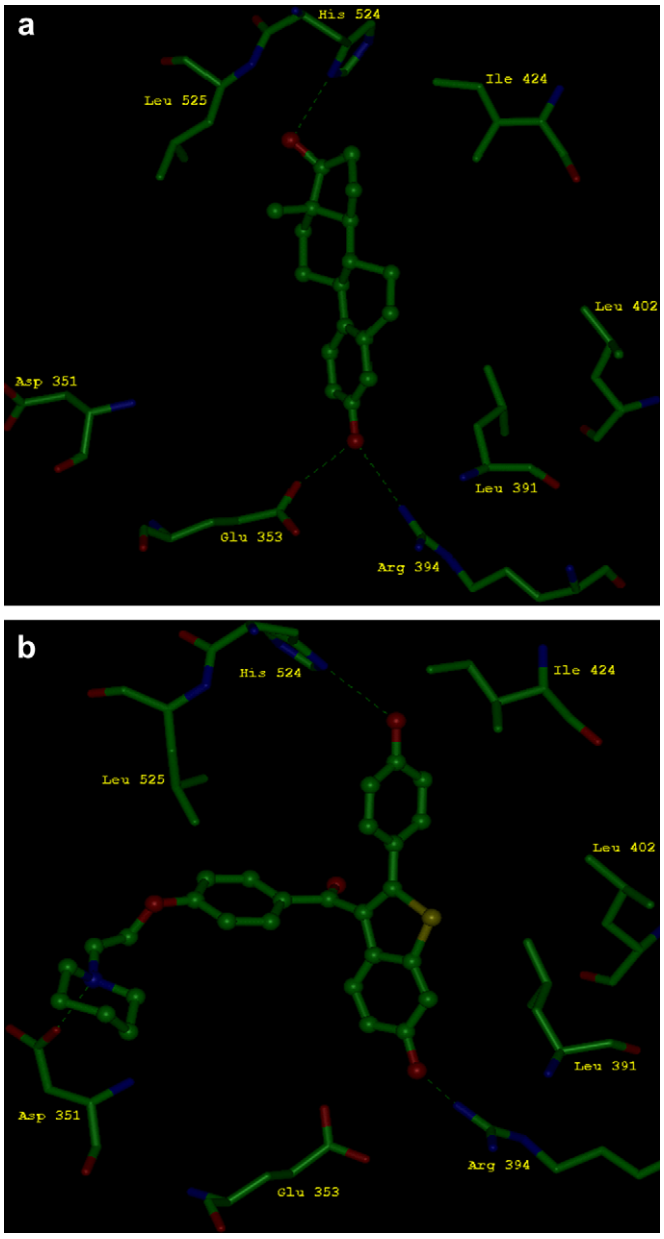



Fig. 3. Docked estradiol (a) and raloxifene (b) in the binding pocket of ER (LBD). The H- bonding is shown by dotted lines.

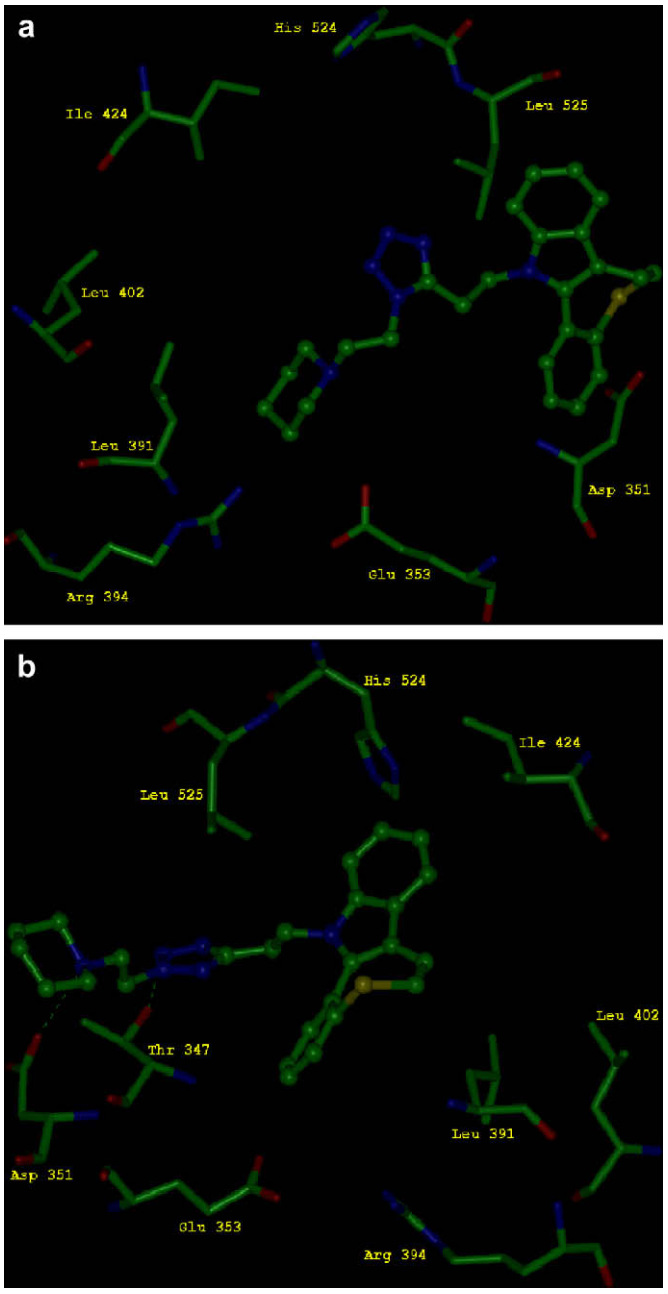



Fig. 4. Amino acid residues involved in the interactions with compounds **4a** (a) and **5a** (b) in the binding site of ER resulting from docking experiments. The binding cavity of ER shows interaction with neighbouring amino acids through hydrogen bonding (dotted lines).

comparable to raloxifene. The tetracyclic core is analogously aligned like estradiol or raloxifene. The phenyl ring of tetracyclic core shares binding pocket with His524 lying in close proximity, but the binding pocket of Glu353 and Arg394 is shifted a little away because of the puckering of seven membered ring giving an upward twist to the benzothiepine core. The side chain projects towards Asp351 making contact with the nitrogen of the piperidine ring, also the tetrazole ring shows interaction with another amino acid Thr347. The binding pockets comprising the salient amino acids are conserved and this could explain the marginally better receptor binding and moderate antagonism of *N*-2 regioisomers than *N*-1 regioisomers.

## 5. Conclusions

In summary, we have explored some new indole derivatives by introducing for the first time 5-substituted *N*-ethyl amino tetrazolyl moiety in place of conventional phenoxy alkyl amino basic side chain as potential estrogen antagonists for contraceptive activity. We envisaged promising activity for *N*-2 regioisomers showing favorable crystal structure spatial conformation comparable to known antagonists and supported by molecular docking studies. However, display of weak antagonism by these isomers may be explained due to the larger

size of the side chain resulting in its twisting and thereby reducing the binding affinity to the active sites within the receptor cavity, the absence of hydroxyl groups on the tetracyclic core and steric hindering of an active conformational change of the receptor–ligand complex due to puckering of 7-membered of benzothiepine ring. These studies give credence to the view that formation of desired active conformation at a ''given position in space'' is essential requisite for ligands to show the desired biological effect. More work through modification of chain length is in progress to generate active antagonists.

## 6. Experimental

### 6.1. General

Melting points were taken in open capillaries on an electrically heated melting point apparatus Complab and are uncorrected. IR spectra were recorded on Perkin–Elmer RX-1 spectrophometer using KBr pallets. The FAB spectra were recorded using a beam of Argon (2–8 eV) on Joel SX 102/DA-6000 mass spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded on Bruker DPX-200 (at 200 MHz for $^1$H and at 50 MHz for $^{13}$C) or DRX-300 (at 300 MHz for $^1$H and at 75 MHz for $^{13}$C) spectrometers using $CDCl_3$ as solvent. Tetramethylsilane served as an internal standard in $^1$H NMR and $CDCl_3$ in $^{13}$C spectra. Silica gel (60–120 mesh) was used for column chromatography while silica gel (230–400 mesh) was used for flash chromatography. TLC was run either on precoated silica gel 60F 254 and RP-18 F 254 (Merck) or hand-made plates. Detection of spots was done either by iodine vapors or spraying with 1% ceric sulfate in1 M $H_2SO_4$ followed by heating at 100 °C.

### 6.2. Synthesis of compounds

#### 6.2.1. 3-(6,7-Dihydro-5-thia-12-aza-dibenzo [a,e] azulen-12-yl)-propionitrile (**2a**)

Compound **1a** (251 mg, 1 mmol) was mixed with acrylonitrile (1 ml) and the solution cooled in ice bath. A crystal of resorcinol was added to prevent polymerization. Triton B (0.04 ml, 40% V/V, 0.25 mmol) was added dropwise with stirring when a vigorous reaction set in and the solution became viscous. It was allowed to subside and the mixture was then heated to reflux on a steam bath for 2.5 h. The solution was cooled, extracted with dichloromethane (20 ml × 3), washed with water (10 ml × 2) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give the desired nitrile, which was purified by column chromatography and recrystallised from benzene/hexane. The pure nitrile was obtained, 252 mg (83%); m.p.: 127 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 2913, 2836, 2250, 1592, 1462, 1356; $^1$H NMR ($CDCl_3$): $\delta$ 2.40 (t, 2H), 2.99 (t, 2H), 3.52 (t, 2H), 4.58 (t, 2H), 7.79 (d, $J = 7.5$ Hz, 1H, ArH), 7.61 (d, $J = 7.4$ Hz, 1H, ArH), 7.52–7.17 (m, 6H, ArH); FAB-MS (*m/z*): 304 [M$^+$].

#### 6.2.2. 3-(6,7-Dihydro-5-oxa-12-aza-dibenzo[a,e] azulen-12-yl)-propinonitrile (**2b**)

Yield: 81%; m.p.: 120 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 2926, 2852, 2253, 1594, 1457, 1349; $^1$H NMR ($CDCl_3$): $\delta$ 2.54 (t, 2H), 3.06 (t, 2H), 4.56 (t, 2H), 4.66 (t, 2H), 7.56–7.23 (m, 8H, ArH); FAB-MS (*m/z*): 289 [M$^+ + 1$].

#### 6.2.3. 3-(6,7-Dihydro-5H-benzo [6,7] cyclohepta [1,2-b] indol-12-yl)-propionitrile (**2c**)

Yield: 90%; m.p.: 132 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 2926, 2852, 2252,1594,1457, 1352; $^1$H NMR ($CDCl_3$): $\delta$ 2.28 (m, 2H), 2.53 (t, 2H), 2.56 (t, 2H), 2.65 (t, 2H), 4.61 (t,2H), 7.51 (d, $J = 7.5$ Hz, 1H, ArH), 7.42–7.22 (m, 7H, ArH); FAB-MS (*m/z*): 286 [M$^+$].

#### 6.2.4. 12-[2-(1-H-Tetrazol-5-yl)-ethyl]-6,7-dihydro-12 H-5-thia-12-aza-dibenzo[a,e]azulene (**3a**)

A mixture of compound **2a** (304 mg, 1 mmol), sodium azide (195 mg, 3 mmol), dimethyl formamide (5 ml) and ammonium chloride (214 mg, 4 mmol) was heated in an oil bath for 12 h at 125 °C. Excess solvent was removed under reduced pressure. The residue obtained was dissolved in water (20 ml) and carefully acidified with conc. hydrochloric acid to pH 2. The solution was cooled in ice bath (5 °C), the precipitated solid was filtered and recrystallised from ethanol, 225 mg (65%); m.p.: 152 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3428, 2916, 2732, 1592, 1459, 1349, 1275, 1181, 1109; $^1$H NMR ($CDCl_3$): $\delta$ 2.71 (t, 2H), 3.34 (t, 2H), 3.86 (t, 2H), 4.71 (t, 2H), 7.88 (d, $J = 8.1$ Hz, 1H, ArH), 7.51–7.39 (m, 4H, ArH), 7.13–7.05 (m, 3H, ArH); FAB-MS (*m/z*): 347 [M$^+$]. Anal. calcd for $C_{19}H_{17}N_5S$: C, 65.70; H, 4.89; N, 20.17%; found: C, 65.86; H, 5.01; N, 20.24%.

#### 6.2.5. 12-[2-(1-H-Tetrazol-5-yl)-ethyl]-6,7-dihydro-12 H-5-oxa-12-aza-dibenzo[a,e]azulene (**3b**)

Yield: 73%; m.p.: 162 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3455, 2952, 2885, 1567, 1489, 1341, 1255, 1159, 1115; $^1$H NMR ($CDCl_3$): $\delta$ 2.56 (t, 2H), 3.07 (t, 2H), 4.57 (t, 2H), 4.63 (t, 2H), 7.58 (d, $J = 7.3$ Hz, 1H, ArH), 7.56–7.21 (m, 7H, ArH); FAB-MS (*m/z*): 331 [M$^+$]. Anal. calcd for $C_{19}H_{17}N_5O$: C, 68.82; H, 5.13; N, 21.14%; found: C, 68.99; H, 5.21; N, 21.20%.

#### 6.2.6. 12-[2-(1H-Tetrazol-5-yl)-ethyl]-5,6,7,12-tetrahydro-benzo [6,7] cycloheptal [1,2-b] indole (**3c**)

Yield: 72%; m.p.: 148 °C; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3416, 2930, 2855, 1593, 1463, 1350, 1250, 1181, 1115; $^1$H NMR ($CDCl_3$): $\delta$ 2.21 (m, 2H), 2.40 (t, 2H), 2.59 (t, 2H), 3.22 (t, 2H), 4.74 (t, 2H), 7.53 (d, $J = 7.2$ Hz, 1H, ArH), 7.35–7.05 (m, 7H); FAB-MS (*m/z*): 329 [M$^+$]. Anal. calcd for $C_{20}H_{19}N_5$: C, 72.94; H, 5.77; N, 21.27%; found: C, 73.31; H, 5.89; N, 21.32%.

*6.2.7. 12-{2-[1-(2-Piperidin-1-yl-ethyl)-2H-tetrazol-5-yl]-ethyl}-6,7-dihydro-12H-5-thia-12-aza-dibenzo [a,e]azulene (**4a**)*

Compound **3a** (347 mg, 1 mmol) was dissolved in dry acetone (15 ml) and to the solution was added 1-(2-chloroethyl) piperidine hydrochloride (256 mg, 1.5 mmol) and triethyl amine(151 mg, 5 mmol) and the solution was refluxed for 16 h. The white solid of triethyl ammonium chloride precipitated in the reaction mixture was filtered, and the filtrate obtained was concentrated. The crude residue on purification by column chromatography gave two products: first compound **4a** obtained as colourless crystals, yield: 37%; m.p.: 72 °C; IR (KBr) $\nu_{max}$ ($cm^{-1}$): 2930, 2863, 2365, 1597, 1462, 1352, 1263, 1164; $^1H$ NMR ($CDCl_3$): $\delta$ 1.26 (m, 6H), 2.41 (m, 4H), 2.83 (t, 2H), 2.92 (t, 2H), 3.16 (t, 2H), 3.46 (t, 2H), 4.62 (t, 2H), 4.81 (t, 2H), 7.75 (d, $J = 7.0$ Hz, 1H, ArH), 7.58–7.18 (m, 7H, ArH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 21.95 ($CH_2$), 22.53 ($CH_2$), 22.65 ($CH_2$), 24.54 ($CH_2$), 40.81 ($CH_2$), 41.87 ($CH_2$), 43.42 ($CH_2$), 53.30 ($CH_2$), 56.53 ($CH_2$), 108.62 (CH), 116.38 (C), 116.93 (CH), 119.06 (CH), 121.71 (CH), 126.42 (C), 126.60 (CH), 127.08 (CH), 128.29 (CH), 133.89 (C), 134.12 (C), 134.42 (C), 134.88 (C), 135.88 (CH), 151.82 (C); FAB-MS (*m/z*): 459 [$M^+ + 1$]; HRMS ($ESI^+$) *m/z* calcd for $C_{26}H_{30}N_6S$: 458.2253; found: 458.2247 and a second compound, *12-{2-[2-(2-piperidin-1-yl-ethyl)-1H-tetrazol-5-yl]-éthyl}-6,7-dihydro-12H-5-thia-12-aza-dibenzo[a,e] azulene (**5a**)*, as oily liquid which solidified on standing, yield: 36%; m.p.: 125 °C; IR (KBr) $\nu_{max}$ ($cm^{-1}$): 2929, 2372, 1592, 1459, 1350, 1159; $^1H$ NMR ($CDCl_3$): $\delta$ 1.43 (m, 6H), 2.34 (t, 6H), 2.76 (t, 2H), 3.08 (t, 2H), 3.45 (t, 2H), 4.53 (t, 2H), 4.60 (t, 2H), 7.68 (d, $J = 7.4$ Hz, 1H, ArH), 7.53 (d, $J = 7.5$ Hz, 1H, ArH), 7.41–7.09 (m, 6H, ArH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 23.28 ($CH_2$), 24.42 ($CH_2$), 26.18 ($CH_2$), 30.11 ($CH_2$), 42.87 ($CH_2$), 44.14 ($CH_2$), 50.83 ($CH_2$), 54.76 ($CH_2$), 57.55 ($CH_2$), 110.52 (CH), 117.01 (C), 118.58 (CH), 120.33 (CH), 122.91 (CH), 127.90 (C), 128.09 (CH), 128.60 (CH), 129.80 (CH), 135.62 (C), 136.03 (C), 136.15 (CH), 137.31 (C), 137.50 (C), 163.72 (C); FAB-MS (*m/z*): 459 [$M^+ + 1$]; HRMS ($ESI^+$) *m/z* calcd for $C_{26}H_{30}N_6S$: 458.2253; found: 458.2243.

The two compounds were identified as *N*-1 and *N*-2 alkylated tetrazoles by spectroscopic data and X-ray crystallographic studies.

*6.2.8. 12-{2-[1-(2-Piperidin-1-yl-ethyl)-2H-tetrazol-5-yl]-ethyl}-6,7-dihydro-12H-5-oxa-12-aza-dibenzo [a,e]azulene (**4b**)*

Yield: 42%; oil; IR (Neat) $\nu_{max}$ ($cm^{-1}$): 2945, 2366, 1595, 1451, 1352, 1242, 1175; $^1H$ NMR ($CDCl_3$): $\delta$ 1.25 (m, 6H), 2.00 (t, 4H), 2.36 (t, 2H), 3.04 (m, 4H), 3.53 (t, 2H), 4.56 (t, 2H), 4.88 (t, 2H), 7.56 (d, $J = 6.6$ Hz, 1H, ArH), 7.41 (d, $J = 7.4$ Hz, 1H, ArH), 7.26–7.15 (m, 4H, ArH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 24.30 ($CH_2$), 24.92 ($CH_2$), 26.15 ($CH_2$), 30.11 ($CH_2$), 42.92 ($CH_2$), 44.88 ($CH_2$), 54.86 ($CH_2$), 58.50 ($CH_2$), 77.93 ($CH_2$), 110.35 (CH), 115.22 (C), 118.78 (CH), 120.80 (CH), 123.23 (CH), 123.66 (CH), 124.63 (CH), 126.05 (C), 128.17 (C), 128.52 (CH), 129.26 (CH), 133.80 (C), 138.21 (C), 153.57 (C), 157.62 (C); ESI-MS (*m/z*): 442 [$M^+ + 1$]; HRMS ($ESI^+$) *m/z* calcd for $C_{26}H_{30}N_6O$: 442.2481; found: 442.2448.

*6.2.9. 12-{2-[2-(2-Piperidin-1-yl-ethyl)-1H-tetrazol-5-yl]-ethyl}-6,7-dihydro-12H-5-oxa-12-aza-dibenzo [a,e]azulene (**5b**)*

Yield: 45%; m.p.: 136 °C; IR (KBr) $\nu_{max}$ ($cm^{-1}$): 2928, 2856, 2364, 1591, 1492, 1165, 1117; $^1H$ NMR ($CDCl_3$): $\delta$ 1.25 (m, 2H), 1.54 (m, 4H), 2.43 (m, 4H), 2.85 (t, 2H), 3.04 (t, 2H), 3.28 (t, 2H), 4.57 (t, 2H), 4.62 (t, 2H), 4.71 (t, 2H), 7.57 (d, $J = 7.6$ Hz, 1H, ArH), 7.46 (d, $J = 7.7$ Hz, 1H, ArH), 7.33–7.13 (m. 6H, ArH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 24.42 ($CH_2$), 24.96 ($CH_2$), 26.17 ($CH_2$), 26.52 ($CH_2$), 43.48 ($CH_2$), 50.80 ($CH_2$), 54.77 ($CH_2$), 57.54 ($CH_2$), 78.02 ($CH_2$), 110.51 (CH), 114.20 (C), 118.72 (CH), 120.36 (CH), 122.81 (CH), 123.51 (CH), 124.38 (CH), 126.63 (C), 128.17 (C), 128.68 (CH), 128.88 (CH), 134.33 (C), 138.37 (C), 157.73 (C), 163.70 (C); FAB-MS (*m/z*): 442 [$M^+ + 1$]; HRMS ($ESI^+$) *m/z* calcd for $C_{26}H_{30}N_6O$: 442.2481; found: 442.2443.

*6.2.10. 12-{2-[1-(2-Piperidine-1-yl-ethyl)-1H-tetrazol-5-yl]-ethyl}-5,6,7,12-tetrahydro-benzo [6,7] cycloheptal [1,2-b] indole (**4c**)*

Yield: 39%; oil; IR (Neat) $\nu_{max}$ ($cm^{-1}$): 2933, 2816, 2367, 1597, 1461, 1352, 1160 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 1.28 (m, 4H), 2.69 (m, 4H), 2.45 (t, 2H), 2.52 (t, 2H), 2.66 (t, 2H), 3.09 (t, 2H), 3.73 (t, 2H), 4.84 (t, 2H), 7.61 (d, $J = 6.6$ Hz, 1H, ArH), 7.33–7.16 (m, 7H, ArH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 22.72 ($CH_2$), 24.22 ($CH_2$), 24.37 ($CH_2$), 27.89 ($CH_2$), 31.36 ($CH_2$), 32.59 ($CH_2$), 40.95 ($CH_2$), 46.89 ($CH_2$), 52.37 ($CH_2$), 55.36 ($CH_2$), 108.26 (CH), 114.21 (C), 116.47 (CH), 117.99 (CH), 120.23 (CH), 124.44 (CH), 125.96 (CH), 126.32 (CH), 128.45 (CH), 130.84 (C), 133.96 (C), 135.68 (C), 140.52 (C), 161.23 (C); ESI-MS (*m/z*): 441 [$M^+ + 1$]; HRMS ($ESI^+$) calcd for $C_{27}H_{32}N_6$: 440.2689; found: 440.2663.

*6.2.11. 12-{2-[2-(2-Piperidine-1-yl-ethyl)-1H-tetrazol-5-yl]-ethyl}-5,6,7,12-tetrahydro-benzo [6,7] cycloheptal [1,2-b] indole (**5c**)*

Yield: 40%; oil; IR (Neat) $\nu_{max}$ ($cm^{-1}$): 2929, 2370, 1596, 1459, 1351, 1161; $^1H$ NMR ($CDCl_3$): $\delta$ 1.45 (m, 8H), 2.35 (m, 4H), 2.50 (m, 4H), 2.69 (t, 2H), 4.48 (t, 2H), 4.66 (t, 2H), 7.33 (d, $J = 8.0$ Hz, 1H, ArH), 7.28–7.06 (m, 7H, ArH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 22.62 ($CH_2$), 24.33 ($CH_2$), 24.88 ($CH_2$), 28.41 ($CH_2$), 31.46 ($CH_2$), 32.77 ($CH_2$), 41.01 ($CH_2$), 48.92 ($CH_2$), 53.03 ($CH_2$), 55.70 ($CH_2$), 108.50 (CH), 114.36 (C), 116.95 (CH), 118.22 (CH), 120.45 (CH), 124.93 (CH), 126.04 (CH), 126.45 (C), 128.59 (CH), 131.09 (C), 134.33 (C), 135.87 (C), 140.98 (C), 162.18 (C); ESI-MS (*m/z*): 441 [$M^+ + 1$]; HRMS ($ESI^+$) calcd for $C_{27}H_{32}N_6$: 440.2689; found: 440.2654.

*6.2.12. 12-{2-[1-(2-Pyrrolidin-1-yl-ethyl)-2H-tetrazol-5-yl]-ethyl}-6,7-dihydro-12H-5-thia-12-aza-dibenzo[a,e]azulene (**6a**)*

Yield: 39%; m.p.: 72 °C; IR (KBr) $\nu_{max}$ ($cm^{-1}$): 2926, 2804, 2378, 1594, 1460, 1350, 1229, 1174; $^1H$ NMR ($CDCl_3$):

δ 1.59 (m, 4H), 2.26 (m, 4H), 2.62 (t, 2H), 2.98 (m, 4H), 3.64 (m, 4H), 4.80 (t, 2H), 7.75 (d, $J = 7.6$ Hz, 1H), 7.58–7.16 (m, 7H, ArH); $^{13}$C NMR ($CDCl_3$): δ 23.43 ($CH_2$), 23.87 ($CH_2$), 24.09 ($CH_2$), 42.43 ($CH_2$), 43.78 ($CH_2$), 46.07 ($CH_2$), 54.46 ($CH_2$), 54.97 ($CH_2$), 110.29 (CH), 117.99 (C), 118.63 (CH), 120.76 (CH), 123.42 (CH), 128.01 (CH), 128.37 (C), 128.87 (CH), 129.96 (CH), 135.59 (C), 136.21 (CH), 136.64 (C), 137.43 (C), 153.31 (C); ESI-MS (*m/z*): 445 [$M^+ + 1$]; HRMS ($ESI^+$) *m/z* calcd for $C_{25}H_{28}N_6S$: 444.2096; found: 444.2077.

*6.2.13. 12-{2-[2-(2-Pyrrolidin-1-yl-ethyl)-1H-tetrazol-5-yl]-ethyl}-6,7-dihydro-12H-5-thia-12-aza-dibenzo[a,e]azulene (**7a**)*

Yield: 45%; m.p.: 132 °C; IR (KBr) $\nu_{max}$ ($cm^{-1}$): 2929, 2803, 2365, 1590, 1459, 1172, 1115; $^1$H NMR ($CDCl_3$): δ 1.25 (m, 4H), 2.38 (t, 4H), 2.89 (m, 4H), 3.16 (t, 2H), 3.52 (t, 2H), 4.63 (t, 2H), 4.81 (t, 2H), 7.75 (d, $J = 7.7$ Hz, 1H, ArH), 7.59 (d, $J = 6.4$ Hz, 1H, ArH), 7.45–7.17 (m, 6H, ArH); $^{13}$C NMR ($CDCl_3$): δ 23.25 ($CH_2$), 23.94 ($CH_2$), 26.42 ($CH_2$), 42.84 ($CH_2$), 44.19 ($CH_2$), 52.48 ($CH_2$), 54.46 ($CH_2$), 54.90 ($CH_2$), 110.52 (CH), 116.98 (C), 118.58 (CH), 120.32 (CH), 122.90 (CH), 127.88 (C), 128.10 (CH), 128.62 (CH), 129.79 (CH), 135.57 (C), 136.02 (C), 136.16 (CH), 137.32 (C), 137.46 (C), 163.74 (C); ESI-MS (*m/z*): 467 [$M + Na]^+$; HRMS ($ESI^+$) *m/z* calcd for $C_{25}H_{28}N_6S$: 444.2096; found: 444.2086.

*6.2.14. 12-{2-[1-(2-Pyrrolidin-1-yl-ethyl)-2H-tetrazol-5-yl]-ethyl}-6,7-dihydro-12H-5-oxa-12-aza-dibenzo[a,e]azulene (**6b**)*

Yield: 38%; oil; IR (Neat) $\nu_{max}$ ($cm^{-1}$): 2955, 2810, 2365, 1596, 1461, 1349, 1231, 1173; $^1$H NMR ($CDCl_3$): δ 1.56 (m, 4H), 2.19 (m, 4H), 2.58 (t, 2H), 3.00 (m, 4H), 3.65 (t, 2H), 4.57 (t, 2H), 4.88 (t, 2H), 7.57 (d, $J = 7.7$ Hz, 1H, ArH), 7.41 (d, $J = 7.1$ Hz, 1H, ArH), 7.31–7.13 (m, 6H, ArH); $^{13}$C NMR ($CDCl_3$): δ 22.24 ($CH_2$), 23.17 ($CH_2$), 28.39 ($CH_2$), 41.14 ($CH_2$), 44.46 ($CH_2$), 52.67 ($CH_2$), 53.50 ($CH_2$), 77.56 ($CH_2$), 108.65 (CH), 113.51 (C), 117.10 (CH), 119.12 (CH), 121.55 (CH), 121.98 (CH), 122.93 (CH), 124.45 (C), 126.56 (C), 126.83 (CH), 127.57 (CH), 132.19 (C), 136.56 (C), 151.52 (C), 155.96 (C); ESI-MS (*m/z*): 429 [$M^+ + 1$]; HRMS ($ESI^+$) *m/z* calcd for $C_{25}H_{28}N_6O$: 428.2325; found: 428.2331.

*6.2.15. 12-{2-[2-(2-Pyrrolidin-1-yl-ethyl)-1H-tetrazol-5-yl]-ethyl}-6,7-dihydro-12H-5-oxa-12-aza-dibenzo[a,e]azulene (**7b**)*

Yield: 40%; m.p.: 126 °C; IR (KBr) $\nu_{max}$ ($cm^{-1}$): 2930, 2812, 2363, 1591, 1485, 1173, 1114; $^1$H NMR ($CDCl_3$): δ 1.78 (m, 4H), 2.56 (m, 4H), 3.02 (t, 2H), 3.07 (t, 2H), 3.31 (t, 2H), 4.60 (t, 2H), 4.65 (t, 2H), 4.75 (t, 2H), 7.60 (d, $J = 5.6$ Hz, 1H, ArH), 7.50 (d, $J = 0.48$ Hz, 1H, ArH), 7.48 (d, $J = 2.9$ Hz, 1H, ArH), 7.35–7.17 (m, 5H, ArH); $^{13}$C NMR ($CDCl_3$): δ 22.26 ($CH_2$), 23.26 ($CH_2$), 24.86 ($CH_2$), 41.77 ($CH_2$), 50.74 ($CH_2$), 52.78 ($CH_2$), 53.20 ($CH_2$), 77.65 ($CH_2$), 108.84 (CH), 112.49 (C), 117.04 (C), 118.68 (CH), 121.13 (CH), 121.84 (CH), 122.73 (CH), 124.97 (C), 126.46 (C), 127.01 (CH), 127.21 (CH), 132.65 (C), 136.66 (C), 156.02 (C), 162.05 (C); ESI-MS (*m/z*): 451[$M + Na]^+$; HRMS ($ESI^+$) *m/z* calcd for $C_{25}H_{28}N_6O$: 428.2325; found: 428.2333.

*6.2.16. 12-{2-[1-(2-Pyrrolidin-1-yl-ethyl)-2H-tetrazol-5-yl]-ethyl}-5,6,7,12 tetrahydro-benzo [6,7] cycloheptal [1,2-b]indole (**6c**)*

Yield: 40%; oil; IR (Neat) $\nu_{max}$ ($cm^{-1}$): 2931, 2812, 2363, 1596, 1487, 1348, 1260, 1174; $^1$H NMR ($CDCl_3$): δ 1.61 (m, 6H), 2.27 (m. 6H), 2.53 (t, 2H), 2.63 (t, 2H), 3.06 (t, 2H), 3.81 (t, 2H), 4.84 (t, 2H), 7.61 (d, $J = 6.9$ Hz, 1H, ArH), 7.38–7.16 (m, 7H, ArH); $^{13}$C NMR ($CDCl_3$): δ 20.55 ($CH_2$), 23.90 ($CH_2$), 30.11 ($CH_2$), 33.24 ($CH_2$), 34.12 ($CH_2$), 41.86 ($CH_2$), 46.47 ($CH_2$), 54.52 ($CH_2$), 55.20 ($CH_2$), 110.04 (CH), 116.59 (C), 118.95 (CH), 120.41 (CH), 122.67 (CH), 126.81 (CH), 128.01 (CH), 128.47 (C), 130.51 (CH), 132.20 (C), 135.82 (C), 142.64 (C), 153.09 (C); ESI-MS (*m/z*): 427 [$M^+ + 1$]; HRMS ($ESI^+$) *m/z* calcd for $C_{26}H_{30}N_6$: 426.2532; found: 426.2521.

*6.2.17. 12-{2-[2-(2-Pyrrolidin-1-yl-ethyl)-1H-tetrazol-5-yl]-ethyl}-5,6,7,12-tetrahydro-benzo [6,7] cycloheptal [1,2-b]indole (**7c**)*

Yield: 41%; oil; IR (Neat) $\nu_{max}$ ($cm^{-1}$): 2930, 2372, 1594, 1462, 1351, 1171, 1116; $^1$H NMR ($CDCl_3$): δ 1.21 (t, 2H), 1.76 (m, 4H), 2.31 (m, 4H), 2.54 (t, 2H), 2.66 (t, 2H), 2.91 (t, 2H), 3.17 (t, 2H), 4.56 (t, 2H), 4.73 (t, 2H), 7.59 (d, $J = 7.2$ Hz, 1H, ArH), 7.43–7.10 (m, 7H, ArH); $^{13}$C NMR ($CDCl_3$): δ 18.97 ($CH_2$), 22.48 ($CH_2$), 25.13 ($CH_2$), 31.69 ($CH_2$), 33.03 ($CH_2$), 41.29 ($CH_2$), 50.87 ($CH_2$), 52.97 ($CH_2$), 53.37 ($CH_2$), 110.22 (CH), 116.03 (C), 118.66 (CH), 119.93 (CH), 122.18 (CH), 126.65 (CH), 127.74 (CH), 127.96 (CH), 128.16 (C), 130.29 (CH), 132.80 (C), 136.03 (C), 137.55 (C), 142.67 (C), 163.86 (C); ESI-MS (*m/z*): 427 [$M^+ + 1$]; HRMS ($ESI^+$) *m/z* calcd for $C_{26}H_{30}N_6$: 426.2532; found: 426.2514.

## 7. Biological screening

### 7.1. Anti-implantation activity

Anti-implantation activity was evaluated in adult male and female rats of Sprague–Dawley strain by the method given in literature [24]. The compounds were administered at 10 mg/kg per oral dose as aqueous gum acacia suspension on day one of post-coitum in 1–7 day schedule. On the 11th day of the test, rats of both the control and treated groups were laparotomised and their uteri examined for implantation sites. The results were considered positive when implantation sites were totally absent in both the uterine horns.

### 7.2. Estrogenic activity

Immature female rats of Sprague–Dawley strain (21 days old, weighing 22–30 g) were bilaterally ovariectomised and after post-operative rest for 7 days were divided into different

groups (each group comprising five rats). Each group received orally the test compound (10 mg, contraceptive dose) once daily for three consecutive days. The control group of animals was treated with the vehicle alone. Increase in the uterine fresh weight, status of vaginal opening and cornification of vaginal epithelium were noted at the time of autopsy, *i.e.* 24 h after the last treatment as parameters for evaluation of estrogen agonist activity. The estrogenic activity was assessed by uterine weight gain expressed as percentage increase as compared to control, which was used as basal value [25].

### *7.3. Antiestrogenic activity*

In antiestrogen assay [25] similarly, immature female rats of Sprague–Dawley strain (21 days old, weighing 22–30 g) were bilaterally ovariectomised and after post-operative rest for 7 days were divided into different groups (each group comprising five rats). Each group received 17α-ethynylestradiol (EE) (0.1 μg in 1.0 μg in olive oil) by subcutaneous route and orally the test compound (10 mg, contraceptive dose) once daily for three consecutive days. One group of rats served as control and received EE and vehicle alone. On day 4, that is, 24 h after last treatment, the animals were autopsied. The uterine tissue was removed, made free of fat and fluid and weighed on a torsion balance. Antiestrogenic activity was computed on the basis of decrease in uterine weight in the experimental animals (receiving test compd + vehicle) as compared to that in the control group receiving EE + vehicle alone. Inhibition was expressed as percent inhibition of estradiol induced increase in uterine wet weight.

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