Molecular Docking Study of Isoxazole Indole Derivatives (B2A2 Series) as Promising Selective Estrogen Receptor Modulators & Anticancer Drugs

Authors
Jayashree Monikanta Iyer[1], Aradhana Khare[2], Jaya Pandey[3], Manish Yadav[4]

Affiliations
1 Amity University, Somathne, Panvel, Mumbai, Maharashtra, India
2 Associate Professor, Atlas Skill Tech University, Mumbai, Maharashtra, India
3 Assistant Professor, Amity University, Lucknow, Uttar Pradesh, India
4 Assistant Professor, Amity University, Somathne, Mumbai, Maharashtra, India

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Correspondence
Jayashree Monikanta Iyer
Amity University
Amity School of Applied Sciences
Somathne, Panvel
Mumbai
410206 Maharashtra
India
jayashreeyr@yahoo.co.in
iyerjayashreemonikanta@gmail.com

ABSTRACT

A series of 7 compounds with isoxazole – indole – γ-resorcylic acid scaffold, segregated into B2 & A2 series, wherein, B2 comprises Compounds: 13, 14, 15 & 16 and A2 comprises Compounds: 10, 11 & 12, on the basis of the variable substituents at the indole, resorcinol and isoxazole end of the scaffold as in Figure: 1, were designed and docked with human estrogen receptor: 1ERRα. The Binding affinity (BA) and the interacting amino acids compared with reference selective estrogen receptor modulators (SERM's) such as Raloxifene, Estradiol, Bazedoxifene, Bisphenol, Genistein, Daidzein, Ormiloxifene, Tamoxifen, 6-hydroxy-naphthalen-2yl-benzo(D)-isoxazol-6-ol(1) using PyRx software and their ADME properties predicted with SWISS ADME online tool. Significant similarities and minor differences in the binding pattern between the key interacting aminoacids such as Arg 394, Glu 353, Asp 351, Leu 346, Leu 525, Trp 383, Phe 404, Ala 350, Leu 387, Met 421 responsible for ER agonist/antagonist affinity found in the binding cavity of a 1 Errα -Bazedoxifene/1 Errα -raloxifene/1 Errα -estradiol docked complex AND 1 Errα -isoxazole-indole- resorcinol docked complex indicate their promising potential to serve as potent ER agonists in bone or ER antagonists against breast cancer and other cancer diseases. The Compounds with highest BA is of the order: BA (A1series) > B1series > / < BA(A2 series) > / = BA (B2 series) exceptions: compounds: 4, 5 of B1 series & compound:13 of B2 series with identical and least BA values.
BA(6) = BA(8) > BA(7) > BA(2) > BA(9) = BA(1) > BA(12) > BA(10) = BA(15) = BA(11) = BA(3) > BA(14) = BA(16) > BA(4) = BA(5) = BA(13).

Introduction

Estrogen ((17β-Estradiol(E2)), Estrogen Receptor, Agonist & Antagonist Mechanism [1]

The naturally occurring endogenous human hormone – Estrogen (17β-Estradiol (E2) and the Estrogen receptors (ER) play a direct role in various estrogen related diseases such as infertility, osteoporosis, breast, ovarian, endometrial, colon, prostrate and other cancers. Estrogen is responsible for maintaining bone health, controls the menstrual cycle of reproductive women, also reduces the risk of coronary heart disease, thus a depletion in the levels of the circulating estrogen directly affects bone health whereas an increase stimulates the uterine endometrium resulting in endome-

trial hyperplasia and cancer. SERMs such as Raloxifene, Tamoxifene, Bazedoxifene, Ormiloxifene are class of drugs known to stimulate estrogenic actions in tissues such as the liver, bone and cardiovascular system. They block estrogen action where stimulation is not desirable, such as in the breast and the uterus [2] by mimicking estrogen (17β-estradiol) and blocking it from binding to ER's, thus preventing tumor growth. Tamoxifen is marketed as Nolvadex. It acts as antagonist in breast by selectively blocking estrogen in breast. It acts as an ER agonist in bone and thus prevents osteoporosis. It acts as a partial agonist in the endometrium, thereby increases the risk of uterine cancer). Raloxifene is marketed as Evista. It acts as a partial agonist [3] of the ERα in bone and the liver and antagonist with antiestrogenic activity in breasts [4] and uterus. It also acts as a pure antagonist of Erβ [5, 6]. Bazedoxifeneacetate (1H-indo-5-ol,1-[[4-[2(hexahydro-1H-azepin-1-yl)ethoxy] methyl]2-(-4-hydroxyphenlyl)-3-methyl;] acetic acid) is a part of the marketed combination drug Duavee for prevention of postmenopausal osteoporosis. It is under study for possible treatment of breast cancer and pancreatic cancer [7, 8]. It has absolute bioavailability of 6.2 % which is 3-fold higher than that of Raloxifene.It has agonistic effects on bone and lipid metabolism but not on breast and uterine endometrium [9]. It is well tolerated, displays no increase in hot flush incidences, uterine hypertrophy or breast tenderness [10]. It competitively blocks 17β-estradiol by high and similar binding to both ERα and ERβ [11].

Chemistry

Like Bazedoxifene, the isoxazole- indole-γ-resorcylic acid scaffold compounds: 10,11,12, 13,14,15 & 16 of the present study comprise an indole core, wherein the indole moiety is flanked by an isoxazole 3-COOH group on 1side & a γ-resorcylic acid group on the other. The free 3-OH at Resorcinol end is blocked by Methoxy group(OMe) in compounds 10 to 16 of B2 A2 series whereas compounds: 1 to 9 of B1A1 series of our previous study possess a free 3-OH at Resorcinol end. Pls see ▶ **Fig. 1**.

The isoxazole indole compounds-viz-10,11,12, 13,14,15 & 16 with variable substituents at the indole, resorcinol and isoxazole end were segregated into subparts: B2series and A2 series wherein B denotes N-n butyl substituted indole and A denotes N-unsubstituted indole, 2 denotes substitution with meta OMe at resorcinol end. The B2 series comprises compounds: 13, 14, 15 & 16.The A2 series includes compounds: 10, 11 & 12. The route of synthesis(ROS) of compounds of B2 & A2 series are provided in ▶ **Fig. 2** B2 and ▶ **Fig. 3** A2.

Materials and methods

Materials

The download link for the various free software tools employed are provided in ▶ **Table 1**

Steps in Ligand Preparation

i.) The structure of ligand was sketched in JS Marwin ii.) This was followed by 'Clean 2D' iii.) Then, 'Clean 3D' iv.) Then, 'Aromatise' v.) Then, 'Export download' vi.) Saved as pdb vii.) Opened the pdb structure in Chimera viii.) Clicked on Tools ix.) Edited structure – 'Dock preparation/yes-Add Gaster charges -Yes' since the ligand is not peptide.x.) Saved structure as mol file xi.) Finally saved as pdb.

Steps in Protein Preparation

i.) Downloaded 1ERRα – protein receptor from RSCB PDB database. ii.) Fetched the protein 1err protein in pdb format in Chimera iii.) Selected chain B – deleted it by clicking on 'Actions – atoms/bonds-delete.' iv.) Clicked on 'Tools-structure editing-dock preparation-Add amber charges'. v.) Saved as mol file with a name viz-1err_A.mol vi.) Finally saved as 1err_A.pdb.

Experiment & Detection Method

PyRx is an open-source docking software, uses a combination of several softwares such as AutoDock 4.2 Vina, Mayavi, Open Babel, etc. to perform virtual screening and used in drug discovery to search small molecules libraries to identify structures most likely to bind to a drug target, viz a protein receptor. Suitably substituted isoxazole indole derivatives were designed and sketched using ACD Chemsketch, Marvin JS, Open babel, chimera. The protein receptor 1ERRα was downloaded from RSCB PDB database. The docking was validated by redocking the inbound Ral 600 ligand along with the 10 standard reference ligands and test ligands of study using PyRx. The active binding sites were selected, with following grid configuration: co-ordinates receptor = 1ERR_A_DS.pdbqt; exhaustiveness = 8; center_x = 67.1224531708; center_y = 34.2499930764; center_z = 70.8859625079; size_x = 24.9865640151; size_y = 27.0419895035; size_z = 36.9666568732. The docking results analysed using Discovery Studio 2016 64-bit software and ADME properties predicted with Swiss ADME ▶ **Table 2** provides BA scores of the docked compounds.

Results and discussion

Prediction of absorption distribution metabolism & excretion (ADME)properties: summarised in Tables-3 B2 & 4 A2

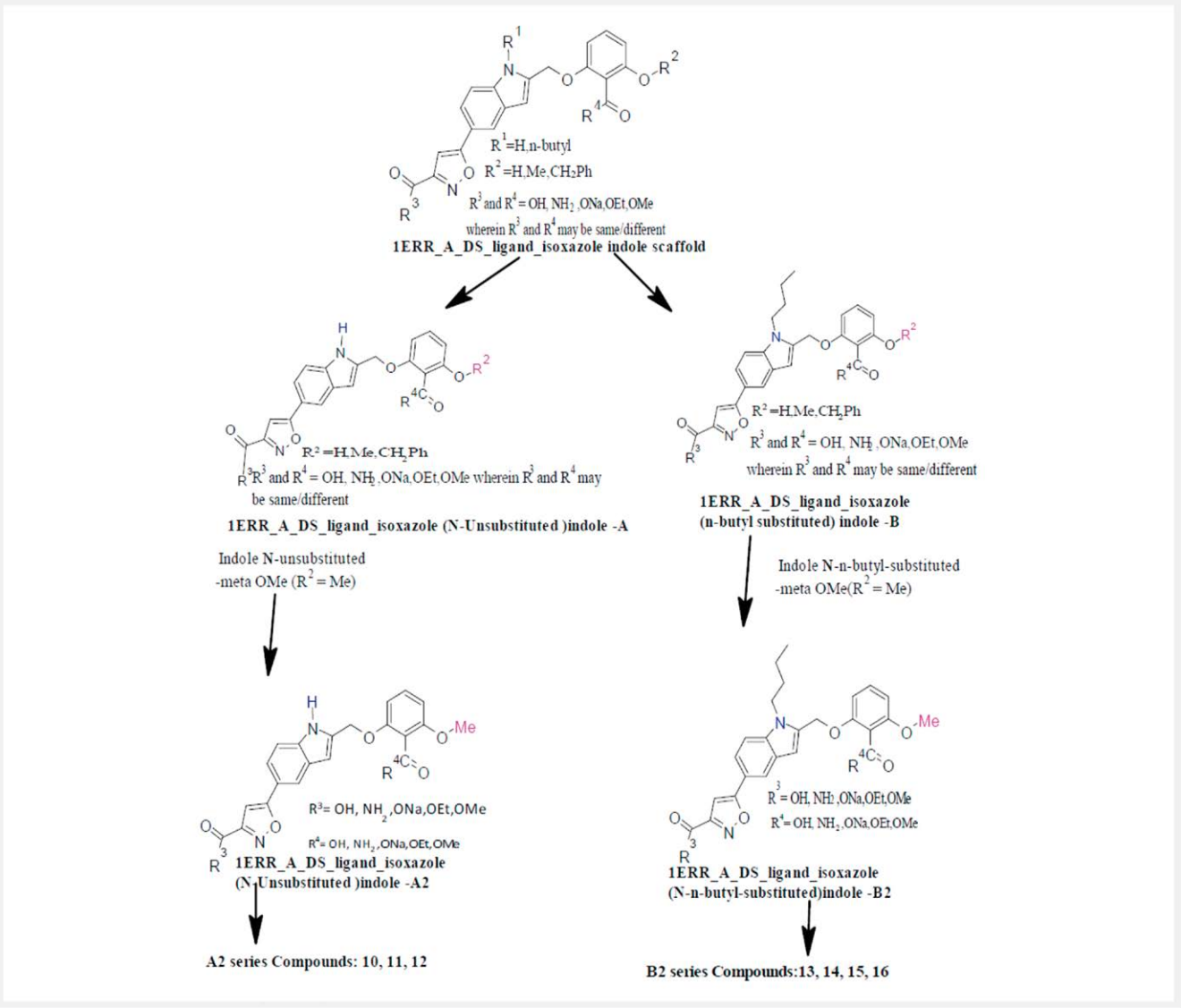
(a.) ClogP

log P value for a compound denoted by $\log_{10}$ PartitionCoefficient (P) = [Organic]/[Aqueous] is defined as the ratio of the compound's organic (oil)-to-aqueous phase concentrations. Optimal physicochemical and ADME for oral drugs likely when optimum logP has a value > 1 < 4. High lipophilicity increases bioavailability by increasing the passage of molecules across cellular barriers resulting in highest intestinal absorption.

ClogP for N-butyl substituted Indole compounds of B2 series (compounds: 13, 14, 15 & 16: ClogP = 3.61, 2.63, 2.85, 2.80) is greater than ClogP for Indole-N-Unsubstituted compounds of A2 series (compounds: 10, 11, 12: ClogP = 1.90, 2.01, 1.72). In contrast to Clog P of N-butyl substituted compounds: 13, 14, 15 & 16 of B2 series; ClogP of compounds: 10, 11 & 12 of A2 series are not influenced much by R3 & R4 substituents.

(b)ClogS

80 % of marketed drugs have logS with an estimated value greater than –4.The range of log S_0 spans from –1.0 to –10.6 (log molar units), averaging at –3.8. Esterification(COOR/COONa salt Group)



▶ **Fig. 1** An overview of isoxazole -indole linked resorcinol compounds docked with 1 ERR-α.

at either of isoxazole/resorcinol end increased Clog S resulting in decreased solubility in both B2 & A2 series. In B2 series: ClogS for compound:13(Di Acid) = –5.11; compound:14(DiCOONa) = –5.62; compound:15(Resorcinol COONa) = –5.36 & compound:16 (Isoxazole COONa) = –5.36.

In A2 series: Clog S for compound:10(Di Acid) = –4.31; compound: 12(Resorcinol COONa) = –4.57& compound:11(Isoxazole COONa) = –4.56.

A2 series compounds have Clog S nearer to –4 and have comparatively higher solubility than B2 series, they are less influenced by substituents R3 & R4 than their B2 counterparts.

(c)logKp

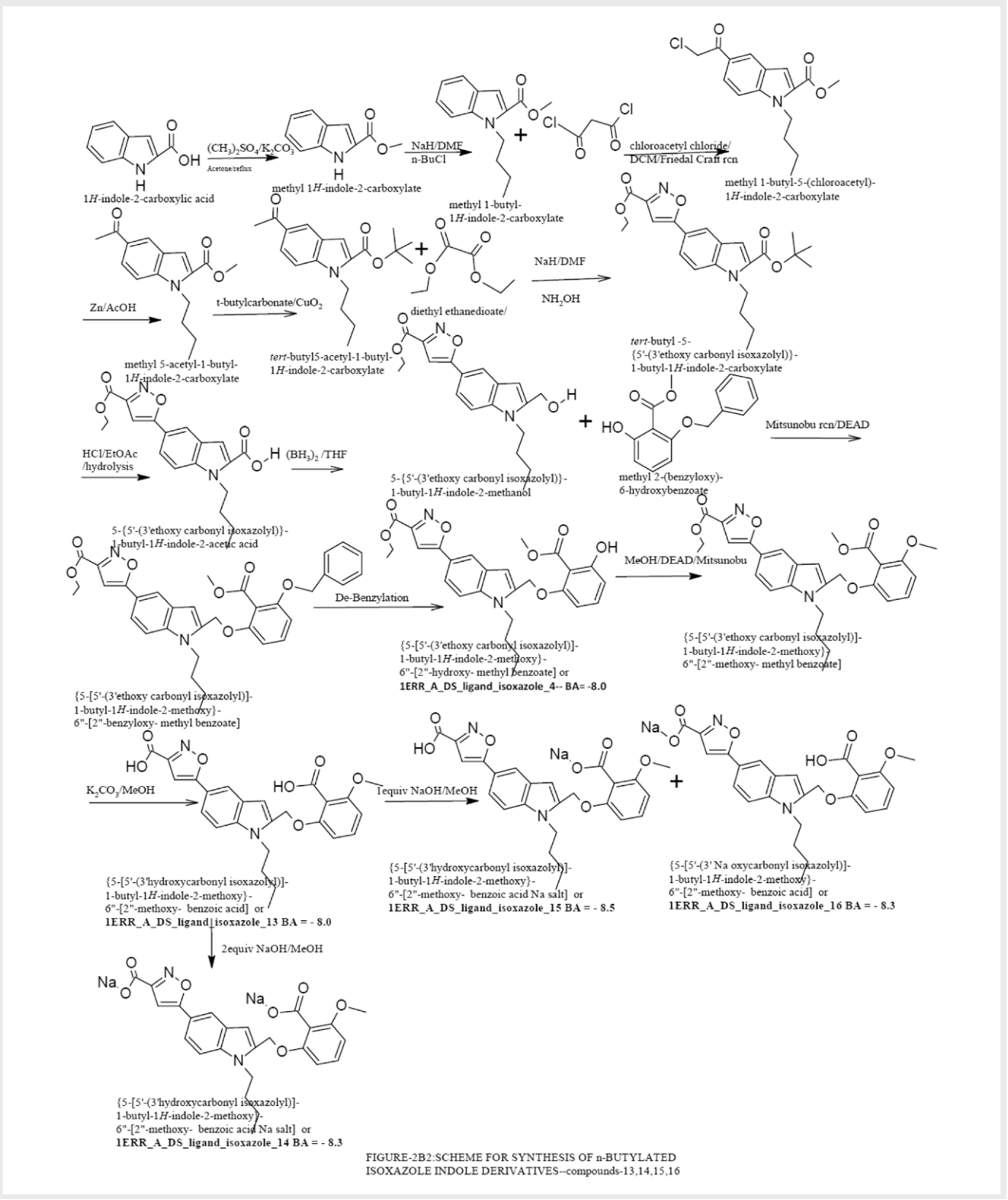
Prediction of permeability coefficient (Kp) for the transport of compounds through mammalian epidermis is based on the linear model by Potts RO. and Guy RH. (1992). The more negative the logKp the less skin permeant the molecule. Esterification increases skin permeability ((ie, -ve values of logkp decreases) whereas amidation decreases skin permeability/logkp (-ve value of logkp increases).

In the B2 series, skin permeability/log Kp increases from –6.19 in diacid compound: 13 and –6.19 in di-Na compound: 14 to –6.10 in mono COONa compound: 15(resorcinol COONa) & –6.10 in mono COONa compound: 16(isoxazole COONa).

In the A2 series, logKp is –6.57 in di-acid compound: 10; –6.56 in mono Na compound: 12 (isoxazole COONa) and –6.48 in mono Na compound: 11(COONa at resorcinol end)

(d):GI absorption, BBB permeability; PGP substrates; CYP inhibitors and other properties

Compounds of both B2 & A2 series obey Lipinski's rule of 5. Many B2 series compounds are predicted to be CYP inhibitors, they have high GI absorption except compound: 13, but not BBB permeant and are PGP substrates except compound 13. Compound:13 has Low GI, not BBB permeant and not PGP substrate. Compounds of A2 series(except compound: 10 which is not CYP inhibitor)are also

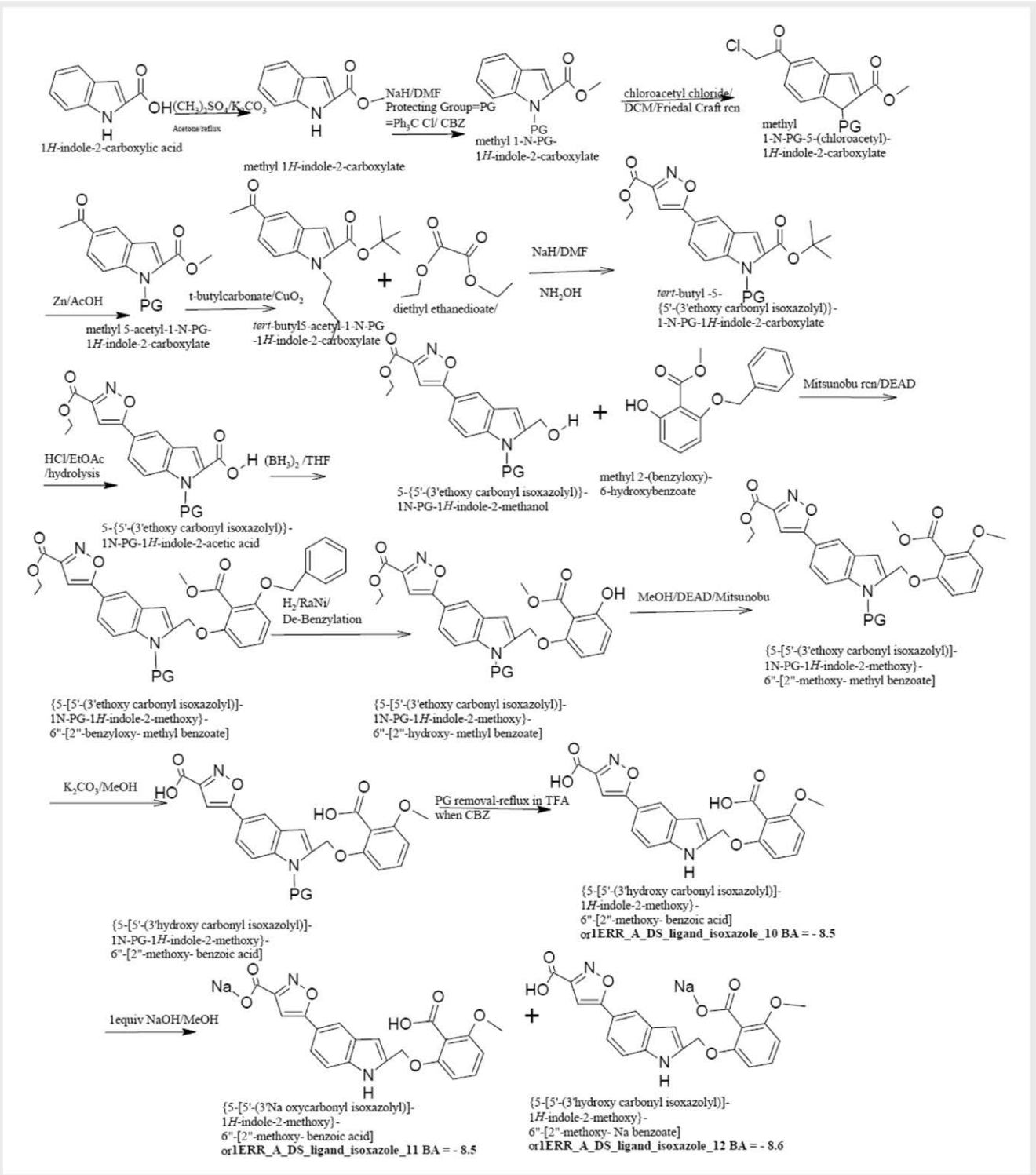


▶ **Fig. 2** B2: ROS of compounds: 13, 14, 15 & 16 of B2 series.

CYP inhibitors, they have high GI absorption, not BBB permeant & are not PGP substrates, except compound: 10 which has high GI absorption & is PGP substrate.

Binding Affinity(BA) score

Binding Affinity(BA) scores of test isoxazole indole compounds and reference standard SERMs are provided in ▶ **Table 2**. Unsubstituted N-Indole compounds of A2 series have higher BA score than



▶ **Fig. 3** A2: ROS of compounds: 10, 11 & 12 of A2 series.

analogous compounds of N-butyl substituted B2 series. N-unsubstituted diacid compound: 10 of A2 series has BA = –8.5 whereas N-butyl substituted analogous diacid compound: 13 of B2series has BA = –8.0. Unlike the BA of N-butyl substituted compounds: 13,14,15 & 16 of B2 series which lie broadly between –8.0 to –8.5, R3 & R4 substituents do not seem to influence much the BA of N-unsubstituted Indole compounds of A2 series: 10,11 & 12 as they lie in the narrow range of –8.5 to –8.6.

Binding Interactions(BI)

▶ **Figs. 4,5** and ▶ **Tables 3,4** provide comparative Binding Interactions(BI) of key amino acids of the test isoxazole indole γ -re-

▶ **Table 1** Link to download various software tools for molecular modeling.

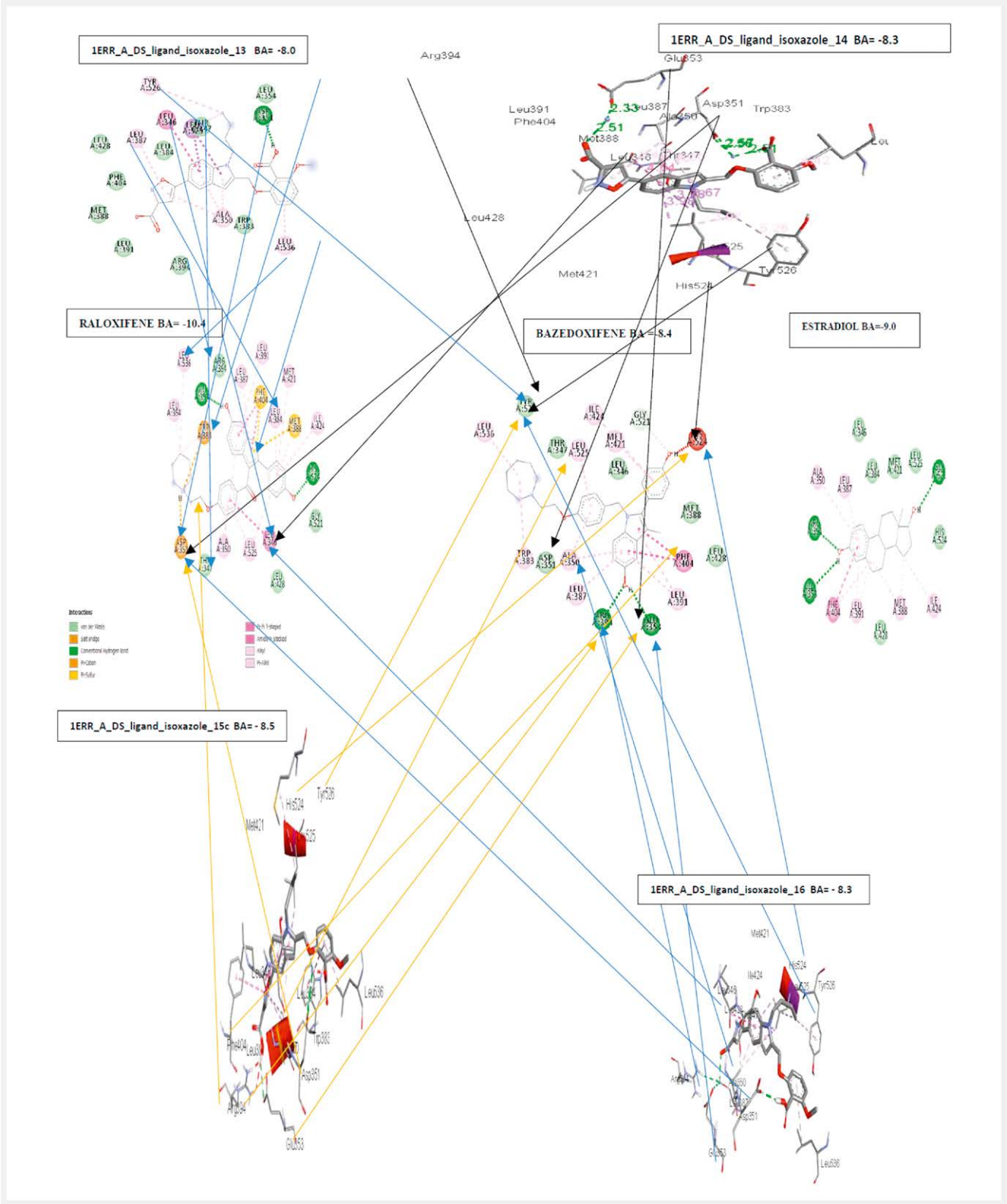
S.N	SOFTWARE TOOLS	LINK
1.	PyRx for VS docking	http://pyrx.sourceforge.net/downloads
2.	ACD CHEMSKETCH	link:https://www.acdlabs.com/resources/freeware/chemsketch/download.php.
3.	OPENBABEL	https://sourceforge.net/projects/openbabel/files/latest/download/
4.	ARVIN JS	https://marvinjs-demo.chemaxon.com/latest/index.html.
5.	UCSFCHIMERA.	https://www.cgl.ucsf.edu/chimera/download.html
6.	DISCOVERY STUDIO	https://discover.3ds.com/discovery-studio-visualizer
7.	SWISS ADME	http://www.swissadme.ch/
8.	RSCB protein database	https://www.rcsb.org/

▶ **Table 2** BA score of B1A1, B2A2 series of compounds vis-a vis standard reference SERMs.

S.N	Ligand	Binding Affinity
1	1ERR_A_DS_Lig_Bazedoxifene_model1	-8.4
2	**1ERR_A_DS_Lig_Bisphenol**	**-8.3**
3	**1ERR_A_DS_Lig_Estradiol**	**-9.0**
4	**1ERR_A_DS_ligand_ormiloxifene_c**	**-9.3**
5	**1ERR_A_DS_Lig_Ral600**	**-11.2**
6	**1ERR_A_DS_Lig_Raloxifene**	**-10.4**
7	**1ERR_A_DS_Lig_Tamoxifen**	**-8.9**
8	**1ERR_A_DS_lig_6-hydroxy-naphthalen-2yl-benzo(D)-isoxazol-6-ol(1)(WAY-397)**	**-7.8**
9	**1ERR_A_DS_lig_Genistein(2)**	**-6.7**
10	**1ERR_A_DS_lig_diadzein(4)**	**-6.6**
11	**1ERR_A_DS_ligand_isoxazole_1-B1series**	**-8.7**
12	**1ERR_A_DS_ligand_isoxazole_2-A1 series**	**-9.2**
13	**1ERR_A_DS_ligand_isoxazole_3-B1 series**	**-8.5**
14	**1ERR_A_DS_ligand_isoxazole_4-B1 series**	**-8.0**
15	**1ERR_A_DS_ligand_isoxazole_5-B1 series**	**-8.0**
16	**1ERR_A_DS_ligand_isoxazole_6-A1 series**	**-9.4**
17	**1ERR_A_DS_ligand_isoxazole_7-A1 series**	**-9.3**
18	**1ERR_A_DS_ligand_isoxazole_8-A1 series**	**-9.4**
19	**1ERR_A_DS_ligand_isoxazole_9-B1 series**	**-8.7**
20	**1ERR_A_DS_ligand_isoxazole_10-A2 series**	**-8.5**
21	**1ERR_A_DS_ligand_isoxazole_11-A2 series**	**-8.5**
22	**1ERR_A_DS_ligand_isoxazole_12-A2 series**	**-8.6**
23	**1ERR_A_DS_ligand_isoxazole_13-B2 series**	**-8.0**
24	**1ERR_A_DS_ligand_isoxazole_14-B2 series**	**-8.3**
25	**1ERR_A_DS_ligand_isoxazole_15-B2 series**	**-8.5**
26	**1ERR_A_DS_ligand_isoxazole_16-B2 series**	**-8.3**

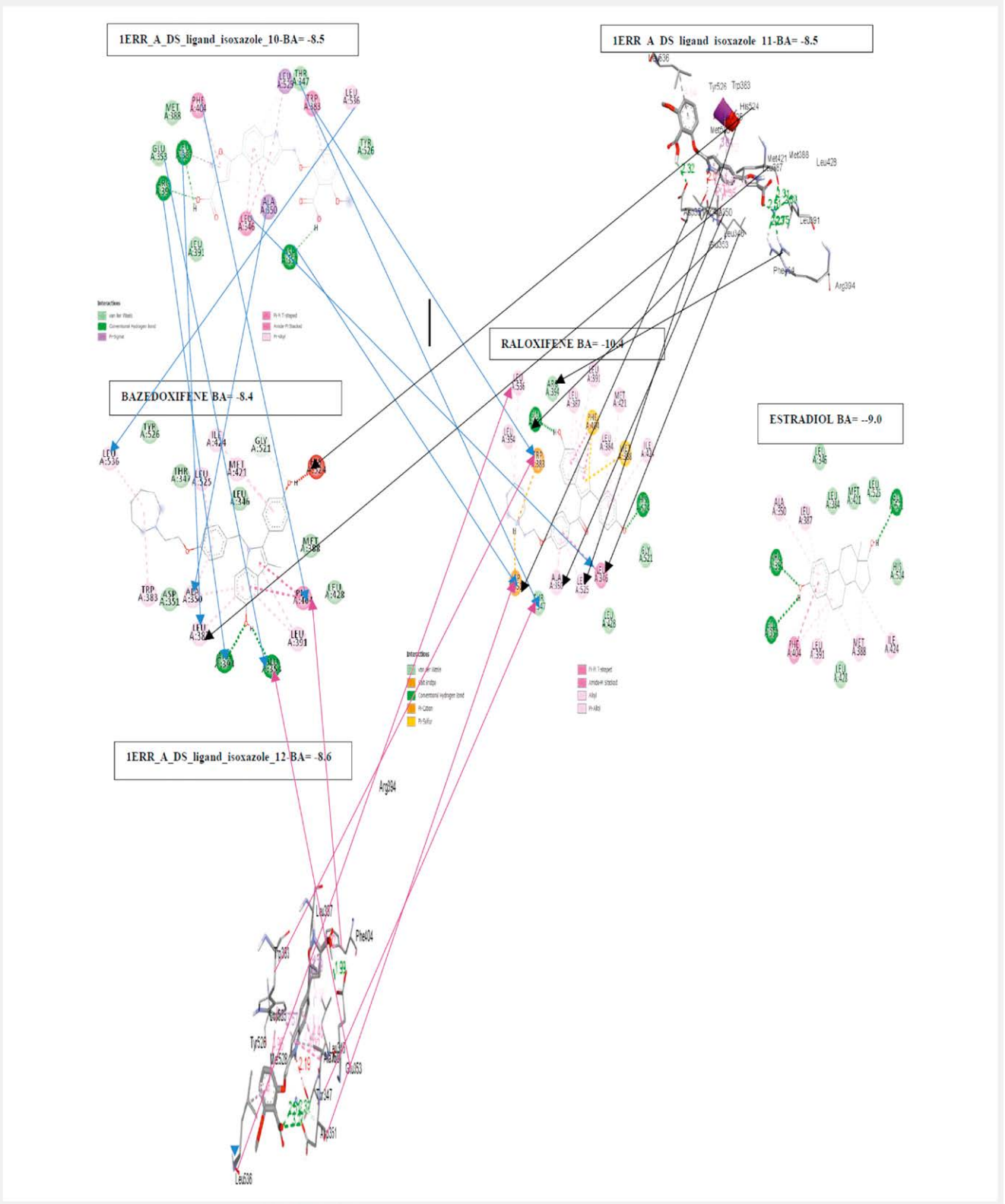
sorcylic acid derivatives of the present study vis a vis standard reference SERMs.

Bazedoxifene (with an indole core) and Raloxifene(with a benzothiophene core), like 4-hydroxytamoxifen(with a stilbene core)-the active metabolite of Tamoxifen(see ▶ **Figs 4** B2, 5 A2), bind to ERα with the hydroxyl group of its phenolic "A ring" through hydrogen bonds with Arg-394 and Glu-353.They form a second hydrogen bond with the help of a second hydroxyl group in their D ring to ER. Ref-[10], Miller et al. teaches the binding of 3-Phenol to be integral in determining agonist activity(e. g.-Agonist binding of Estradiol).The active binding site in the ligand binding domain (LBD) of Erα is composed of hydrophobic residues –3, 6, 7, 8, 11 & 12, wherein amino acid residues 536–544 of Helix 12 determine the agonist/antagonist activity of the ligand [12]. H bond/Electrostatic interactions of Asp 351 with the bulky sidechain of the antagonist are usually present at basic N end (or C end) of the bulky (Piperidinyl 'N'in bazedoxifene/raloxifene) sidechain. It causes effective displacement of Helix-12 & the enclosed hydrophobic amino acid residues 536–544, and forces the antagonist to adopt an orthogonal disposition relative to the core , thereby leading to antagonist activity [13, 14]. Such H bond/Electrostatic interactions of Asp 351 responsible for antagonist activity of Raloxifene and Bazedoxifene, are absent in Tamoxifene & 4-hydroxy tamoxifen.



▶ **Fig. 4** B2: Binding Interactions in B2 series-compounds: 13, 14, 15 & 16 of B2 series.

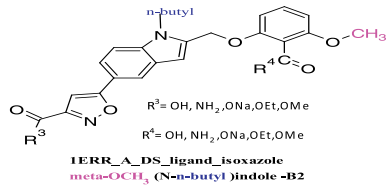
The standard SERMs such as Raloxifene & Bazedoxifene form His-524 H bond through the 2nd OH group in D ring which is unlike that between 17β-estradiol and His-524, as the imidazole ring of His-524 is rotated to counteract the difference of the oxygen position in raloxifene/bazedoxofene and in 17β-estradiol. Wambi et al. [15] attributed the missing H-bond between Bazedoxifene and His

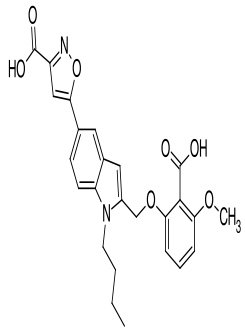
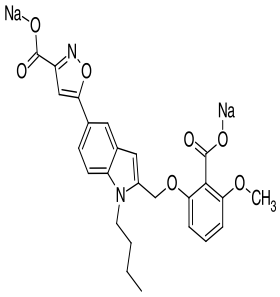
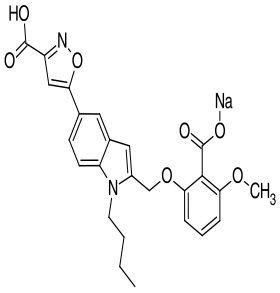
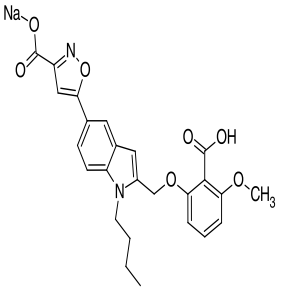


▶ **Fig. 5** A2: Binding Interactions in A2 series- compounds: 10, 11 & 12 of A2 series.

524 in the 4-hydroxy tamoxifen bound receptor to the different orientation of Leu 539 amino acid in the binding site of Bazedoxifene-ER complex as compared to the Raloxifene-ER complex.This triggered a conformational change of helix 12, leading to the recruitment of other proteins by the Bazedoxifene-ER. Wambi et al. concluded that subtle but moderate structural differentiation can dramatically affect the ability of a ligand to regulate cell proliferation and that Bazedoxifene is distinct from other SERMs such as Ra-

▶ **Table 3** B2 SERIES (N-butylated and with OCH3 group) – B2) Indole-N-Butyl-substituted-meta –OMe at Resorcinol end (R^2 = Me)-Compounds-13, 14, 15 & 16.



COMPOUND 13	COMPOUND 14	COMPOUND 15	COMPOUND 16
 1ERR_A_DS_ligand_isoxazole_13 BA = - 8.0	 1ERR_A_DS_ligand_isoxazole_14 BA = - 8.3	 1ERR_A_DS_ligand_isoxazole_15BA - 8.5	 1ERR_A_DS_ligand_isoxazole_16 BA= - 8.3
MF = C25H24N2O7	MF = C25H22N2Na2O7	MF = C25H23N2NaO7	MF = C21H15N2NaO7
MW = 464.47	MW = 508.43	MW = 486.45	MW = 486.45
Canonical Smiles = CCCCn1c(COc2cccc(c2C(=O)O)OC)cc2c1ccc(c2)c1onc(c1)C(=O)O	Canonical Smiles = CCCCn1c(COc2cccc(c2C(=O)O[Na])OC)cc2c1ccc(c2)c1onc(c1)C(=O)O[Na]	Canonical Smiles = CCCCn1c(COc2ccccc(c2C(=O)O[Na])OC)cc2c1ccc(c2)c1onc(c1)C(=O)O	Canonical Smiles = CCCCn1c(COc2cccc(c2C(=O)O)OC)cc2c1ccc(c2)c1onc(c1)C(=O)O[Na]
Heavy Atoms = 34	Heavy Atoms = 36	Heavy Atoms = 35	Heavy Atoms = 35
Ar heavy atoms = 20	Ar heavy atoms = 20	Ar heavy atoms = 20	Ar heavy atoms = 20
Fraction Csp3 = 0.24	Fraction Csp3 = 0.24	Fraction Csp3 = 0.24	Fraction Csp3 = 0.24
Rotatable bonds = 10	Rotatable bonds = 12	Rotatable bonds = 11	Rotatable bonds = 11
H bond acceptors = 8	H bond acceptors = 8	H bond acceptors = 8	H bond acceptors = 8
H bond donors = 2	H bond donors = 0	H bond donors = 1	H bond donors = 1
MR = 124.51/TPSA = 124.02	MR = 121.42/TPSA = 102.02	MR = 122.96/TPSA = 113.02	MR = 122.96/TPSA = 113.02
ADME	ADME	ADME	ADME
Consensus LogP = 3.61	Consensus LogP = 2.63	Consensus LogP = 2.85	Consensus Log P = 2.80
ESOL log S /Silicos -IT log Sw = - 5.11/-6.82	ESOL log S /Silicos -IT log Sw = - 5.62/-7.55	ESOL log S /Silicos -IT log Sw = - 5.36/-7.18	ESOL log S /Silicos -IT log Sw = - 5.36/-7.18
ESOLsolubility/SilicosITmg/mol = 3.61–03/7.10E-05	ESOLsolubility/SilicosITmg/mol = 1.23–03/1.43E-05	ESOLsolubility/Silicos ITmg/mol = 2.11E03/3.18E-05	ESOLsolubility/SilicosIT mg/mol = 2.11E-03/3.18E-05
Moderately Soluble/ Poorly Soluble	Moderately Soluble/ Poorly Soluble	Moderately Soluble/ Poorly Soluble	Moderately Soluble/ Poorly Soluble
logKp(cm/s) = -6.19	logKp(cm/s) = -6.19	logKp(cm/s) = -6.10	logKp(cm/s) = -6.10
GI absorption- Low	GI absorption- High	GI absorption- High	GI absorption- High
BBB permeant-No	BBB permeant-No	BBB permeant-No	BBB permeant-No
Pgp substrate-No	Pgp substrate-Yes	Pgp substrate-Yes	Pgp substrate-Yes
CYP1A2 inhibitor-No	CYP1A2 inhibitor-No	CYP1A2 inhibitor-No	CYP1A2 inhibitor-No
CYP2C19 CYP2C9/CYP2D6 inhibitor- Yes/Yes/No	CYP2C19 CYP2C9/CYP2D6 inhibitor- Yes/Yes/Yes	CYP2C19 CYP2C9/CYP2D6 inhibitor- Yes/Yes/No	CYP2C19 CYP2C9/CYP2D6 inhibitor- Yes/Yes/No
CYP3A4 inhibitor-Yes	CYP3A4 inhibitor-Yes	CYP3A4 inhibitor-Yes	CYP3A4 inhibitor-Yes
Lipinski = 0/Ghose = 0/Veber = 0/Egan = 0/Muegge = 0/Lead likeliness violations = 3	Lipinski = 1/Ghose = 1/Veber = 1/Egan = 0/Muegge = 0/Lead likeliness violations = 3	Lipinski = 0/Ghose = 1/Veber = 1/Egan = 0/Muegge = 0/Lead likeliness violations = 3	Lipinski = 0/Ghose = 1/Veber = 1/Egan = 0/Muegge = 0/Lead likeliness violations = 3
Pain/Brenk alert = 0	Pain/Brenk alert = 0	Pain/Brenk alert = 0	Pain/Brenk alert = 0
BA score = 0.56	BA score = 0.55	BA score = 0.56	BA score = 0.56
Synthethic accessibility = 3.74	Synthethic accessibility = 3.96	Synthethic accessibility = 3.87	Synthethic accessibility = 3.92

▶ **Table 3** (Continued)

INTERACTING AMINO ACIDS	INTERACTING AMINO ACIDS	INTERACTING AMINO ACIDS	INTERACTING AMINO ACIDS
Isoxazole end -O –absent H bond	Isoxazole end -O –absent H bond	Isoxazole end -O –absent H bond	Isoxazole end -O –absent H bond
Isoxazole end—H/OH/COOH with Leu 387 BL = 2.22	Isoxazole end—Ona BL = 2.51 & Na-Glu 353 BL = 2.33	Isoxazole end—H/OH/COOH—H-Leu 387 H BL = 2.79	Isoxazole end—O/ONa/ COONa—ONa H BL = 2.51 & Na-Glu 353 BL = 2.39
NA	NA	IsoxazoleendO/OH/COOH—O_Arg 394 BL = 2.29	NA
Resorcinol end H ofCOOH --1 H with Asp 351 BL = 2.48	Resorcinol end-COONa—Ona BL = 2.51 & Na-Asp 351H bond = 2.56 &Na-Asp C-H bond = 3.29	Resorcinol end-COONa—ONa BL = 2.01-unfavourable & Na-Asp 351 H bond = 2.28 & Na -Asp 351—CH bond = 3.12	Resorcinol end-H/COOH—H-Asp 351 BL = 2.37
Pi-sigma interactions—Indole -Ar scaffold with Leu 525	Pi-sigma interactions—Indole -Ar scaffold with Leu 525	Pi-sigma interactions—Indole -Ar scaffold with Leu 525 & Isoxazole with Leu 387	Pi-sigma interactions—Indole -Ar scaffold with Leu 525
Amide Pi -stacked interactions of Indole-Ar with Leu 346	Amide Pi -stacked interactions of Indole-Ar with Leu 346	Amide Pi -stacked interactions of Indole-Ar with Leu 346	Amide Pi -stacked interactions of Indole-Ar with Leu 346
NA	NA	Pi-Pi T shaped interactions from isoxazole with Phe 404 & from Resorcinol-Ar to Trp383	NA
Pi-Alkyl interactions from Indole scaffold & isoxazole to Ala 350	Pi-Alkyl interactions from Indole & isoxazole scaffold to Ala 350	Pi-Alkyl interactions from Indole& isoxazole scaffold to Ala 350	Pi-Alkyl interactions from Indole& isoxazole scaffold to Ala 350
Pi-Alkyl interactions from Isoxazole -Ar to Leu 387	NA	NA	NA
Pi-Alkyl interactions form n-butyl to Leu 525 & Tyr 526	Pi-Alkyl interactions form n-butyl to Leu 525 & Tyr 526	Alkyl interactions form n-butyl to Met 528	Alkyl interactions form n-butyl to Tyr 526 & Leu 525
Pi-Alkyl interactions form Resorcinol to Leu 536	Pi-Alkyl interactions from Resorcinol-Ar to Leu 536	Pi-Alkyl interactions from Resorcinol-Ar to Leu 536	Pi-Alkyl interactions from Resorcinol-Ar to Leu 536

loxifene, Tamoxifene & 4-hydroxy tamoxifen in its ability to inhibit hormone-independent breast cancer cell growth and to regulate ER and cyclin D1 expression in resistant cells. The isoxazole indole linked to γ resorcylic acid derivatives of the present study exhibited the following features consistent with key requirements for SERM:

(i)Intramolecular H bond interactions between the Isoxazole scaffolds with Arg 394 & Glu 353/Tyr 526&Trp 383/Leu 387 interactions

In the N-butylated isoxazole indole scaffolds of category B2(Compounds: 13, 14, 15 & 16): Arg 394 & Glu 353 are present at the isoxazole end in all except compound 13 wherein Arg 394 is present in binding cavity, but it's interaction is not visible.

In the N-Unsubstituted isoxazole indole scaffolds of category A2(Compounds: 10, 11 & 12), the 3-OH at resorcinol end being blocked by Me group, exhibit a binding pattern similar to the N_ butyl substituted B2 analogs. This is in contrast to its unsubstituted A1 series compounds (2, 6, 7, 8) with Free/unblocked 3-OH at resorcinol end which show a reversal of binding pattern vis-a-vis their N-butylated B1 series analogs (1, 3, 4, 5, 9).

Compounds: 10 & 11 exhibit binding interactions with Arg 394. Glu 353 is present in binding cavity. Compound: 11 also exhibits an additional conventional H bond interaction with Leu 391 at isoxazole end. The presence of Leu 387 H bond/Glu 353 observed along with Arg 394 in some of the docked isoxazole indole scaffolds such as compounds: 13 & 15 of B2 series & compounds: 10 &11 of A2 series at the isoxazole end including standard SERMs such as Raloxifene, Bazedoxifene could be presumed to be due to the proximity to Glu 353.

Consistent with the teachings of Wambi et al., the slight variations in the binding interactions of the present Isoxazole indole scaffold -viz; additional H bond interaction such as a Tyr 526 or Leu 387 interaction can be attributed to moderate and subtle structural differences between Isoxazole indole scaffold and standard SERM's such as Bazedoxifene ,Raloxifene,4-OHT,Estradiol.These differences may dramatically confer increased cell proliferation regulating and cancer inhibiting ability to Isoxazole indole scaffolds of the present study vis a vis other SERMs.

(ii) H Bond/Electrostatic Interactions with Asp 351

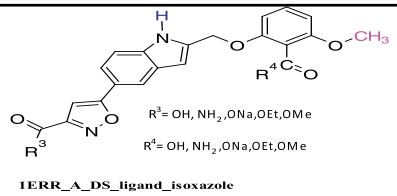
In the N-butylated isoxazole indole compounds: 13, 14, 15 & 16 of the of B2 series, H bond/Electrostatic interactions between Asp 351 & resorcinol end is present at resorcinol end. However, in the N-unsubstituted compounds: 10,11 & 12 of A2 series,H bond/Electrostatic interactions between Asp 351 & resorcinol end is present in compounds: 11 & 12, but absent in compound:10

(iii)Intramolecular H bond interactions with His 524

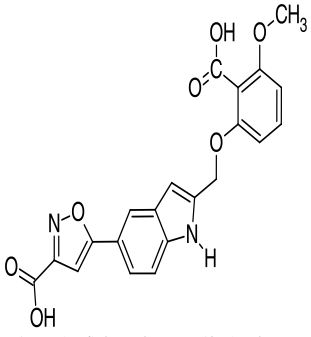
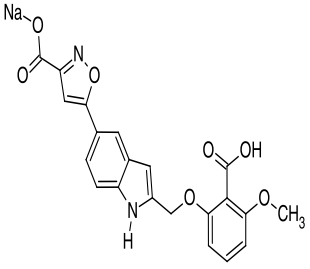
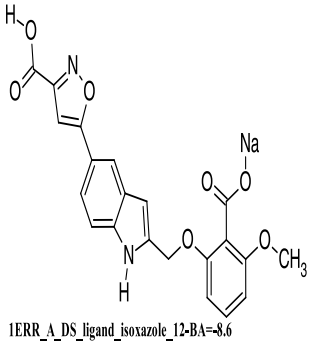
H bond interactions of His 524 was absent in compounds: 13,14,15 & 16 of B2 series.

H bond interactions of His 524 was absent in compounds: 10,11&12 of A2 series.

▶ **Table 4** A2 SERIES (N -unsubstituted indole and with OCH3 group) – A2) INDOLE-N-UNSUBSTITUTED-META –OMe AT RESORCINOL END (R^2 = Me) COMPOUNDS-10,11 &12.



1ERR_A_DS_ligand_isoxazole

COMPOUND 10	COMPOUND 11	COMPOUND 12
 1ERR_A_DS_ligand_isoxazole_10 BA= - 8.5	 1ERR_A_DS_ligand_isoxazole_11 BA= - 8.5	 1ERR_A_DS_ligand_isoxazole_12-BA=-8.6
MF = C21H17N2O7	MF = C21H15N2NaO7	MF = C21H16N2NaO7
MW = 409.37	MW = 430.34	MW = 431.35
Canonical Smiles = COc1cccc(c1C(=O)O)OC[C]1Cc2c(N1)ccc(c2)c1onc(c1)C(=O)O	Canonical Smiles = [Na]OC(=O)c1noc(c1)c1ccc2c(c1)cc([nH]2)COc1cccc(c1C(=O)O)OC	Canonical Smiles = [Na]OC(=O)c1c(OC[C]2Cc3c(N2)ccc(c3)c2onc(c2)C(=O)O)cccc1OC
Heavy Atoms = 30	Heavy Atoms = 31	Heavy Atoms = 31
Ar heavy atoms = 17	Ar heavy atoms = 20	Ar heavy atoms = 17
Fraction Csp3 = 0.19	Fraction Csp3 = 0.1	Fraction Csp3 = 0.19
Rotatable bonds = 7	Rotatable bonds = 8	Rotatable bonds = 8
H bond acceptors = 8	H bond acceptors = 8	H bond acceptors = 8
H bond donors = 3	H bond donors = 2	H bond donors = 2
MR = 107.34/TPSA = 131.12	MR = 103.64/TPSA = 123.88	MR = 105.7/TPSA = 120.12
ADME	ADME	ADME
Consensus Log P = 1.9	Consensus Log P = 2.01	Consensus Log P = 1.72
ESOL log S /Silicos -IT log Sw = - 4.31/-5.58	ESOL log S /Silicos -IT log Sw = - 4.56/-6.46	ESOL log S /Silicos -IT log Sw = - 4.57/5.95
ESOLsolubility/SilicosITmg/mol = 1.99E-02/1.08E-03	ESOLsolubility/SilicosITmg/mol = 1.20E-02/1.48E-04	ESOL solubility /Silicos -ITmg/mol = 1.17E-02/4.85E-04
Moderately Soluble/ Moderately Soluble	Moderately Soluble/ Poorly Soluble	Moderately Soluble/ Moderately Soluble
logKp(cm/s) = -6.57	logKp(cm/s) = -6.56	logKp(cm/s) = -6.48
GI absorption- HIGH	GI absorption- High	GI absorption- High
BBB permeant-No	BBB permeant-No	BBB permeant-No
Pgp substrate-Yes	Pgp substrate-No	Pgp substrate-No
CYP1A2 inhibitor-No	CYP1A2 inhibitor-No	CYP1A2 inhibitor-No
CYP2C19/CYP2C9/CYP2D6 inhibitor- No/No/No	CYP2C19/CYP2C9/CYP2D6 inhibitor- Yes/Yes/No	CYP2C19/CYP2C9/CYP2D6 inhibitor- Yes/Yes/No
CYP3A4 inhibitor-No	CYP3A4 inhibitor-No	CYP3A4 inhibitor-Yes
Lipinski = 0/Ghose = 0/Veber = 0/Egan = 0/Muegge = 0/	Lipinski = 0/Ghose = 1/Veber = 1/Egan = 0/Muegge = 0/	Lipinski = 0/Ghose = 0/Veber = 0/Egan = 0/Muegge = 0/
Lead likeliness violations = 1	Lead likeliness violations = 2	Lead likeliness violations = 2
Pain/Brenk alert = 0	Pain/Brenk alert = 0	Pain/Brenk alert = 0
BA score = 0.56	BA score = 0.56	BA score = 0.56
Synthethic accessibility = 3.67	Synthethic accessibility = 3.74	Synthethic accessibility = 3.80
INTERACTING AMINO ACIDS	INTERACTING AMINO ACIDS	INTERACTING AMINO ACIDS
Isoxazole end -O –absent H bond	Isoxazole end -O –absent H bond	Isoxazole end -O –absent H bond

▶ **Table 4** (Continued)

COMPOUND 10	COMPOUND 11	COMPOUND 12
NA	Isoxazole end—O/ONa/COONa—ONa H BL = 2.51 &	NA
Isoxazole end-- O/H/COOH -Arg 394 BL = 2.31	Isoxazole end—O/ONa/COONa --Na-Arg 394–2 H bonds of BL = 2.33 & 2.75	NA-but Arg 394 present in Binding cavity
Isoxazole end-- H/OH/COOH with Leu 387 BL = 2.02	Isoxazole end—O/ONa/COONa –Na-Leu 387 BL = 2.31 & Na-Leu 391 BL = 2.83	Isoxazole end—H/OH/COOH— H-Glu 353- BL = 1.99
Resorcinol end H of COOH --1 H with Asp 351 BL = 2.11	Resorcinol end-H/COOH—H-Asp 351 BL = 2.32	Resorcinol end—O/ONa/COONa –O-Na BL = 2.51 & O-Asp 351 BL = 1.99 &Na-Asp 351 BL = 2.32 &N-Asp C-H bond BL = 3.45
Indole NH interaction-NA	Indole NH-Thre 347-H bond unfavourable = 2.01	Indole NH-Thre 347-H bond unfavourable = 2.01
Amide Pi -stacked interactions of Indole-Ar with Leu 346	Amide Pi -stacked interactions of Indole-Ar with Leu 346	Amide Pi -stacked interactions of Indole-Ar with Leu 346 & isoxazole with Phe 404
Pi-sigma interactions—Indole -Ar scaffold with Leu 525 & Isoxazole-Ar to Leu387	Pi-sigma interactions—Indole -Ar scaffold with Leu 525	Pi-sigma interactions—Indole -Ar scaffold with Leu 525 & Isoxazole with Leu 387
Pi-Pi-T shaped interactions of Isoxazole-Ar with Phe 404& Resorcinol-Ar scaffold with Trp383	NA	Pi-Pi T shaped interactions from Resorcinol-Ar to Trp383 & Isoxazole -Ar with Ph404
Pi-Alkyl interactions from Indole scaffold to Leu 525, Leu 346 & Ala 350	Pi-Alkyl interactions from Indole scaffold to Ala 350	Pi-Alkyl interactions from Indole scaffold to Ala 350
Pi-Alkyl interactions from Isoxazole -Ar to Ala350	Pi-Alkyl interactions from Isoxazole -Ar to Ala 350	Pi-Alkyl interactions from Isoxazole -Ar to Ala 350
Pi-Alkyl interactions from Resorcinol-Ar to Leu 536	Pi-Alkyl interactions from Resorcinol-Ar to Leu 536	Pi-Alkyl interactions from Resorcinol-Ar to Leu536

(iv)Tyr 526 H bond interactions at the > C = O end of isoxazole moiety

H bond interactions with Tyr 526 at Isoxazole end were absent in B2 & A2 series. This is similar to compounds of B1& A1 series except Compounds 7 & 8 of A1 series wherein H bond interactions with Tyr 526 at Isoxazole end were present.

(v) Met 421, Phe 404, Leu 536,Leu 525 interactions

Met 421 in human ER has been suggested to be involved in binding interactions with the aliphatic and/or polar functional groups at the C-16 and C-17 positions of an estrogen [16]· Sigma bond interactions with Met 421 was observed in Compounds 1, 3, 4, 5 & 9 of A1 series only. Wen Ming Li [17] concluded from his study of molecular dynamics for the ER–Diethylstilbesterol and ER–4-hydroxy tamoxifen complexes:

(a) The residue of Glu353 participates in both agonist and antagonist recognition.
(b) His524 residue only takes part in the agonist recognition.
(c) Met343, Leu346, Thr347 and Asp351 residues might only take part in the antagonist recognition.
(d) Leu346 and Thr347 were found to be important in antagonist recognition.

- **Met 421 sigma bond interaction & His 524 H bond interaction at resorcinol end:** Unlike the compounds: 1, 3, 4, 5 & 9 of B1 series & compounds: 2, 6, 7 & 8 of A1 series [18], Met 421 sigma bond interaction aswell as His 524 H bond interaction at Resorcinol end are absent in compounds : 13, 14, 15 & 16 of B2 series & compounds: 10, 11 & 12 of A2 series.
- **H bond interaction with Thre 347 at 'O'** of isoxazole moiety were found to be absent in compounds 13, 14, 15 & 16 of B2 series and Compound 10 of A2 series, unlike compounds: 1, 3, 4, 5 & 9 of B1 series & compounds:2, 6, 7 & 8 of A1 series [18].

H bond interaction with Thre 347 at 'O' of isoxazole moiety were found to be present as unfavourable in compounds: 11 & 12 of A2 series.

(vi) Sigma interaction with Leu 525

Sigma interaction with Leu 525 was found to be present in all compounds of B2 & A2 series similar to compounds of B1 & A1 series. Compound: 15 of B2 series showed an additional sigma bond interaction with Leu 387 at isoxazole end.

(vii) Amide Pi stacked interaction with Leu 346

Similar to compounds of B1A1 series [18], Amide Pi stacked interaction with Leu 346 was found in all the compounds 10,11, & 12 of A2 series aswell as compounds 13,14,15 & 16 of B2 series.

(viii) PiPi T interactions with Phe 404 at isoxazole end & Trp 383 at Resorcinol end

Similar to compounds of A1 series and unlike B1 series [18]; Pi Pi T interactions with Phe 404 at isoxazole end & Trp 383 at Resorcinol end were absent in B2 series, except compound:15; but present in compounds:10 & 12 of A2 series, except compound:11.

(ix).Pi Alkyl interactions

Similar to compounds of B1A1 series [18], Pi alkyl interactions of Ala 350, Leu 525 at Indole end were found to be present in compounds: 13, 14, 15 & 16 of B2 series & 10, 11 & 12 of A2 series.

(x).Pi alkyl interactions of Leu 428 & ILeu424/Leu 536 at Resorcinol end

Unlike the compounds : 1,3 , 4 & 9 except 5 of B1 series which showed presence of Pi alkyl interactions of Leu 428 & ILeu424 at Resorcinol end, and similar to compound: 2, 6, 7, & 8 of A1 series including compound 5 of B1 series [18], the present study compounds: 13, 14, 15 & 16 of B2 series and compounds: 10, 11 & 12 of A2 series showed Pi alkyl interaction with Leu 536 at Resorcinol end.

(xi) Pi alkyl interactions with Leu 387, Leu 391 & Phe 404 at n butyl end

Pi alkyl interactions with Leu 387, Leu 391 & Phe 404 at n butyl end were found to be present in compounds: 1, 3, 4, 5 & 9 of B1 series, but absent in A1 series [18].

Unlike compounds: 1, 3, 4, 5 & 9 of B1 series [18], n butyl alkyl interactions with Leu 525 & Tyr 526 were present in the compounds: 13, 14 & 16 of B2 series, whereas compound:15, n- butyl interactions with Met 528 was present.

Conclusion

Significant similarities and minor differences in the binding pattern of key interacting aminoacids found in the binding cavity of a 1 Errα -Bazedoxifene/1 Errα -raloxifene/1 Errα -estradiol complex and responsible for ER agonist/antagonist affinity such as Arg 394,Glu 353, Asp 351, Leu 346, Leu 525, Trp 383, Phe 404, Ala 350, Leu 387, Met 421 and the test isoxazole indole scaffold compounds of the present study indicate the promising potential of suitably substituted isoxazole indole derivatives of the present study to serve as potent ER agonists/inhibitors against breast cancer and other cancer diseases. A reversal of the binding pattern found in the Free/unblocked 3-OH series: B1(N-butylated compounds-1, 3, 4, 5, & 9) & A1(N-unsubstituted compounds –2, 6, 7 & 8) was absent in compounds of B2 series: 13, 14 ,15 & 16) vis a vis compounds of A2 series: 10, 11 & 12 due to the presence of blocking 3-OMe group at the resorcinol end.

Compounds of A2 series were found to have comparatively higher BA and better ADME profile than the N-butylated Indole B2 counterparts. The Compounds with highest BA is of the order: BA (A1series) > B1series > / < BA(A2 series) > / = BA (B2 series) exceptions: compounds: 4, 5 of B1 series & compound: 13 of B2 series with identical and least BA values. BA(6) = BA(8) > BA(7) > BA(2) > BA(9) = BA(1) > BA(12) > BA(10) = BA(15) = BA(11) = BA(3) > BA(14) = BA(16) > BA (4) = BA(5) = BA(13). Further clinical studies need to be done to evaluate further potential for research and development.

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Conflicts of Interest

The authors report no conflicts of interest.

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