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Investigating the Anti-Oestrogenic Effects of Ephedrine and *De novo* Design of Oestrogen Modulating Molecules

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ABSTRACT
Ephedrine is a thermogenic compound extracted from the shrub plant *Ephedra sinica* which has enjoyed worldwide use as the active ingredient in weight loss products prior to being banned from use by the FDA in 2004 following numerous adverse effect reports including cardiac arrest. A study published by Arbo *et al.* in 2008 hypothesised the potential anti-oestrogenic effects of ephedrine following a series of *in vivo* uterotrophic assays in immature female rats. Through this study we report the validation of this hypothesis by means of comparison of *in silico* ligand binding affinities of the isomers of ephedrine with that of 17-*β*-oestradiol. Furthermore the ephedrine scaffold was used as a pharmacophore for the *de novo* creation of high affinity novel ligands for the oestrogen receptor on the basis that the absence of the steroidal scaffold and the use of a hitherto unutilised pharmacophoric platform would mitigate many of the adverse effects associated with 17-*β*-oestradiol analogs and also those of the Selective Oestrogen Receptor Modulators in contemporary use.

Keywords: Ephedrine, oestrogen receptor, antioestrogen, *de novo* drug design.

INTRODUCTION

Ephedrine, a natural thermogenic compound derived from plants of the genus *Ephedra*, was identified by Arbo *et al.* as possessing anti-oestrogenic effects. This hypothesis was made subsequent to a series of uterotrophic assays carried out on immature female rats [1]. This finding instigated this study which sought to validate Arbo *et al.*'s finding through *in silico* modelling. This was considered important owing to the fact that positive correlations with Arbo *et al.*'s assertion would indicate that this molecule could be potentially detrimental to female adolescent sexual development and function. From a drug design perspective, this study was carried out since it was postulated that ephedrine which is non-steroidal and chemically unrelated to known ligands of the oestrogen receptor (ER) could have potential in the identification of lead molecules which could serve as antagonists for this receptor. This hypothesis gains importance when it is kept in mind that antagonists and partial agonists of this receptor have found clinical use in the management of breast cancer and osteoporosis.

Consequently, this study aimed to evaluate the *in silico* ligand binding affinity (LBA) of the ephedrine isomers for the ER and to compare this with that of 17-*β*-oestradiol in order to be able to make predictions regarding competitive displacement of 17-*β*-oestradiol by ephedrine in an *in vivo* scenario. Furthermore, the ephedrine scaffold was modelled based on the bound coordinates of 17-*β*-oestradiol, and used as a scaffold for the *de novo* design of high affinity ligands for the ligand binding pocket of the oestrogen receptor (ER_LBP) such that these could be further modelled into clinically useful molecules for the management of breast cancer and osteoporosis.

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METHODOLOGY

Protein Data Bank [2] crystallographic deposition 1ERE [3] describing the bound coordinates of 17-*β*-oestradiol with the ER, resolved to 3.10 Å was used as a template for this study. Molecular modelling was carried out using Sybyl®-X v.1.1. LBA estimations were performed using X-Score v.1.2 and *de novo* design was carried out using LigBuilder v.1.2.

The trimeric crystallographic deposition 1ERE was reduced to a single monomer and all co-crystallised water molecules at a radius ≥ 5Å from the ER_LBP were removed. The 17-*β*-oestradiol molecule was extracted from its cognate ligand binding pocket and saved, preserving its bioactive bound coordinates, in mol2 format. The now *apo*-ER was saved in pdb format. File format selection was carried out based on the requirements of X-Score v.1.2, whose algorithm was used for the *in silico* evaluation of the LBA (p*K*d) of this small molecule for its receptor.

The four diastereoisomers of ephedrine *(1R,2S)-(-)-ephedrine, (1S,2R)-(+)-ephedrine, (1R,2R)-(-)-pseudoephedrine and (1S,2S)-(+)-pseudoephedrine* were constructed and minimised in Sybyl®-X v.1.1. These were

saved in mol2 format and docked into the ER_LBP via the ligand similarity suite algorithm in Sybyl®-X v.1.1 using the bioactive coordinates of 17-*β*-oestradiol as templates for docking. The molecules were allowed conformational rotation within a static ER_LBP and the twenty-one highest affinity conformers were identified in each case. The ligand binding energy (LBE) in kcal mol^{-1} was measured using the Tripos force field in Sybyl®-X v.1.1. Graphs of *in silico* LBA (p*K*d) and LBE (kcal mol^{-1}) against conformer number were plotted for each diastereoisomer considered, and the conformations with the optimal combination of high LBA (p*K*d) and low LBE (kcal mol^{-1}) were identified in each case, the premise being that those would be high affinity stable structures (Fig. 2).

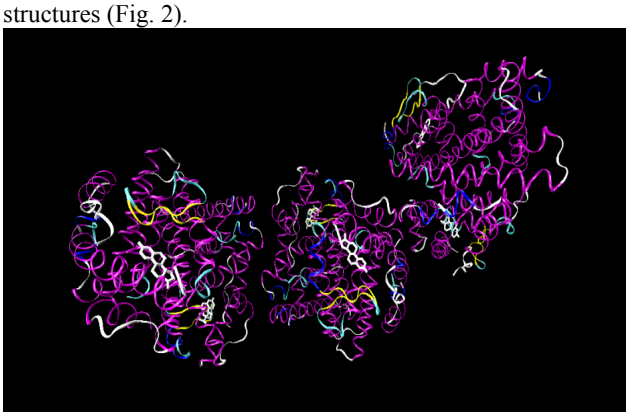
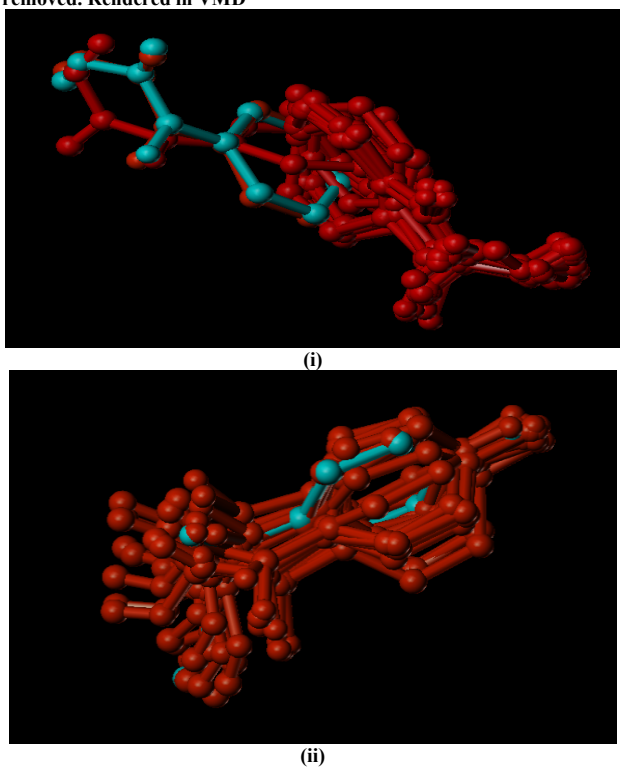


Fig. 1: The *holo*- trimeric ER with co-crystallised water molecules removed. Rendered in VMD®

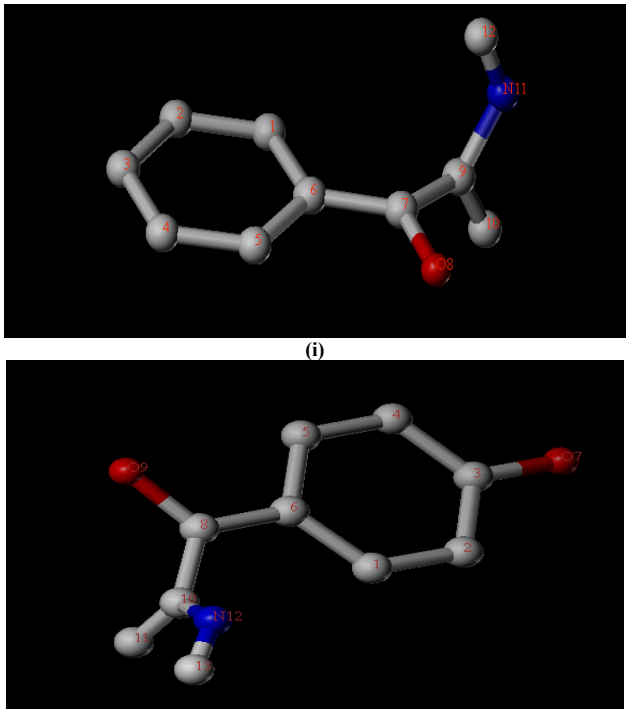


(i)

(ii)

Fig. 2: (i) The selected conformer for the unsubstituted *R,S*-ephedrine isomer highlighted in cyan among the 21 generated conformers; (ii) the selected conformer for the 3-hydroxyl substituted *R,S*-ephedrine isomer highlighted in cyan among the 21 generated conformers
Rendered in Sybyl®-X v.1.1

Literature indicates [4] that the presence of a hydroxyl group at position three of the A-ring in 17-*β*-oestradiol is essential for oestrogenic activity. Consequently a hydroxyl group was sketched at this position on the benzene ring of the ephedrine scaffold (Fig. 3ii) for each diastereoisomeric structure, the twenty-one highest affinity conformations identified and their corresponding LBEs (kcal mol^{-1}) were once more calculated. Graphs of LBA (p*K*d) and LBE (kcal mol^{-1}) were also generated for the 3-hydroxyl substituted ephedrine diastereoisomers.



(i)

(ii)

Fig. 3: (i) The selected conformer for the unsubstituted *R,S*-ephedrine isomer ; (ii) the selected conformer for the 3-hydroxyl substituted *R,S*-ephedrine isomer
Rendered in Sybyl®-X v.1.1.

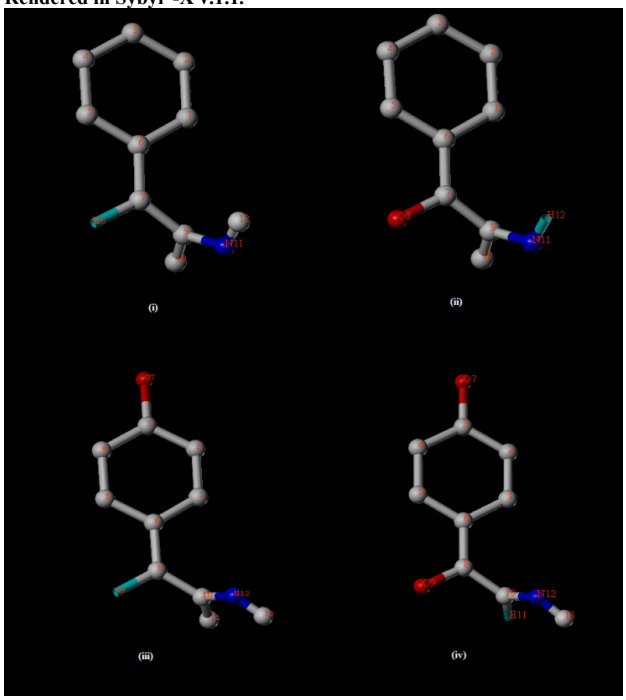
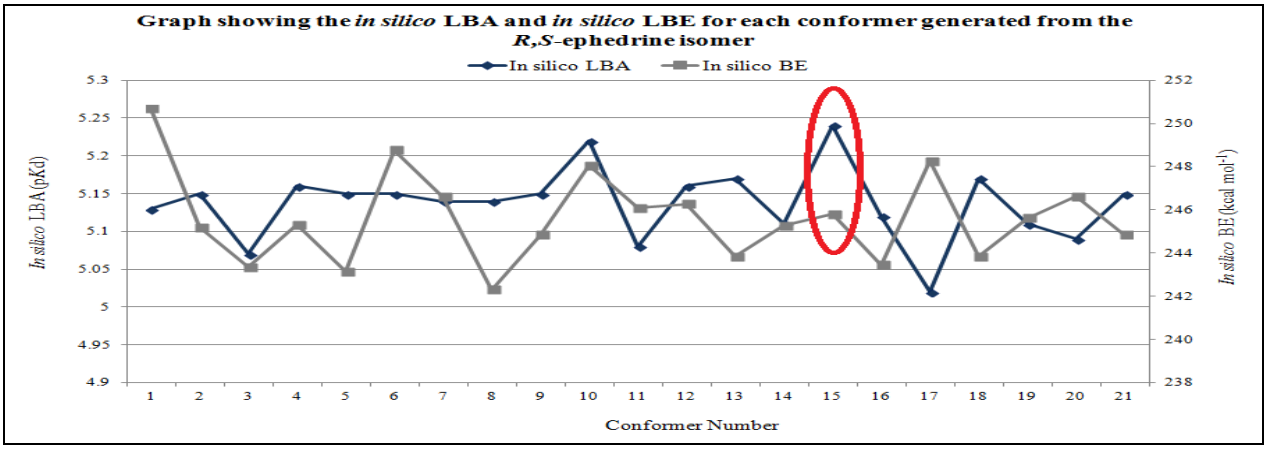
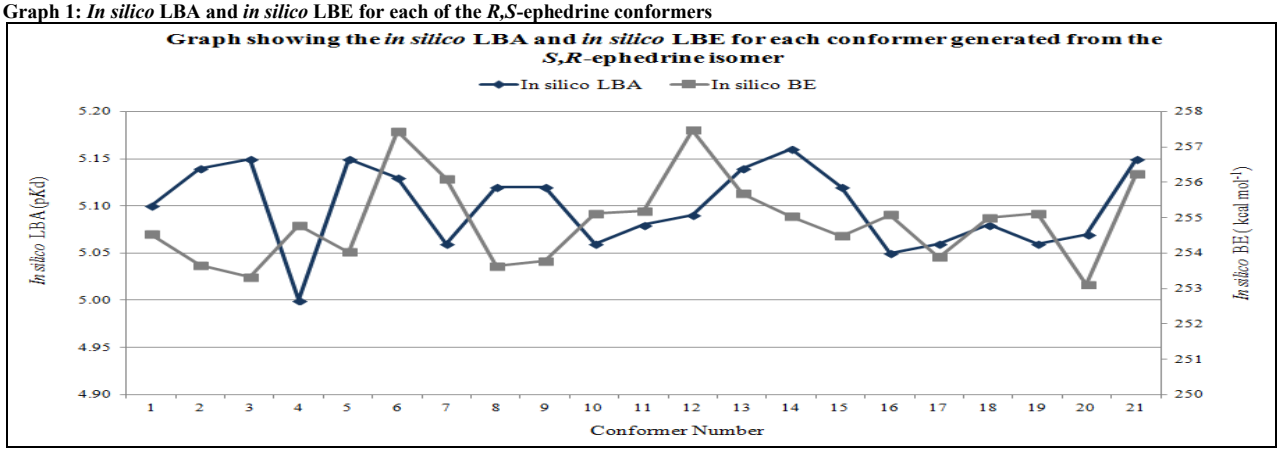


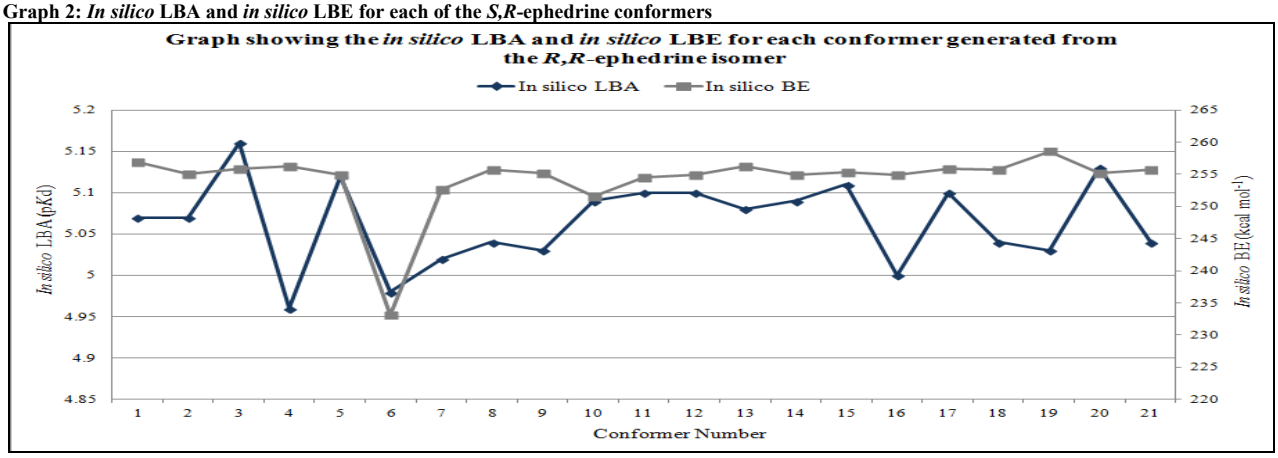
Fig. 4: A representation depicting the four seed structures. Seeds A and B were derived from the coordinates of the unsubstituted conformer number 15 while seeds C and D were derived from the coordinates of the 3-hydroxy substituted conformer number 13.
Rendered in Sybyl®-X v.1.1.



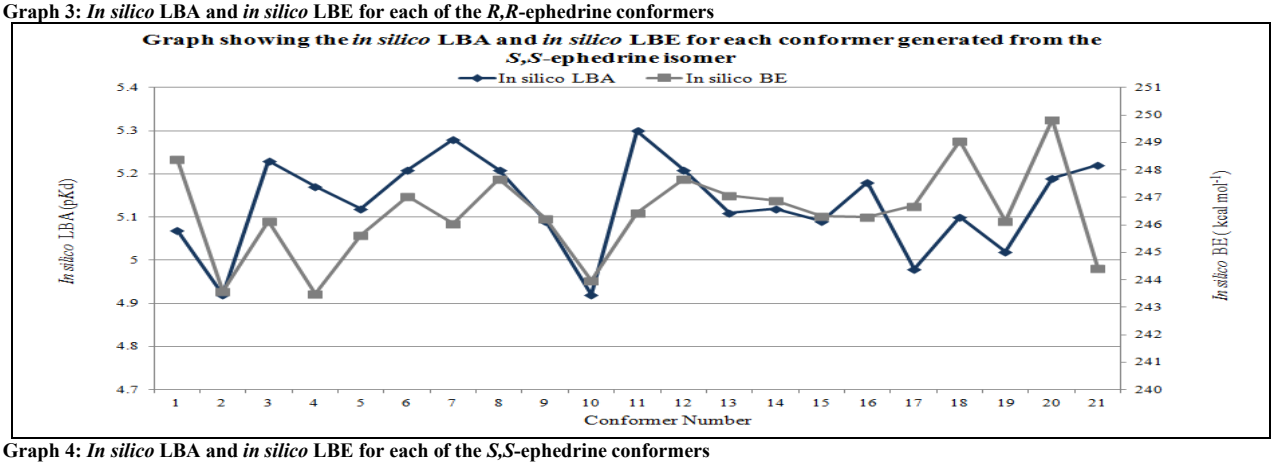
Graph 1: *In silico* LBA and *in silico* LBE for each of the *R,S*-ephedrine conformers



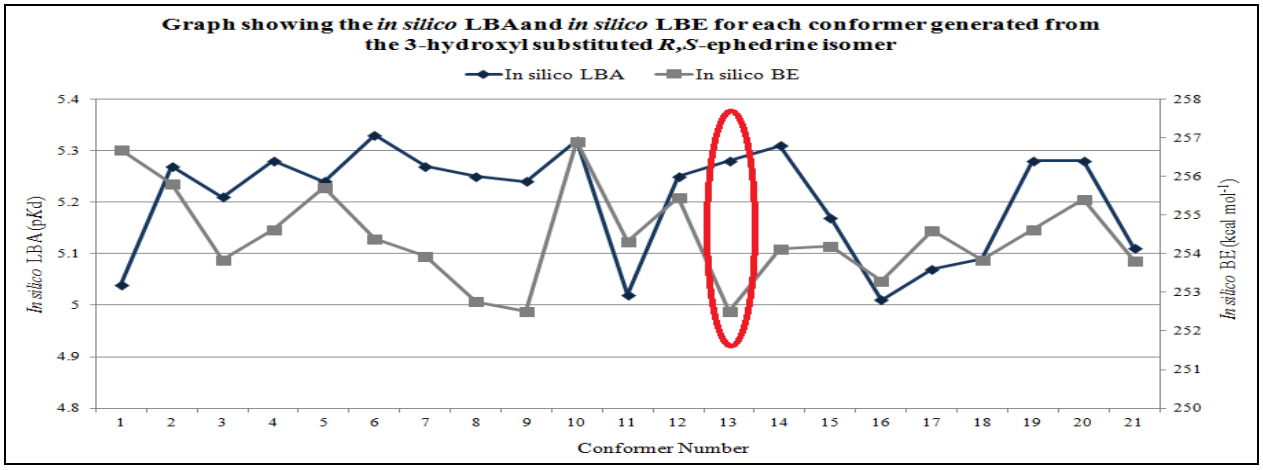
Graph 2: *In silico* LBA and *in silico* LBE for each of the *S,R*-ephedrine conformers



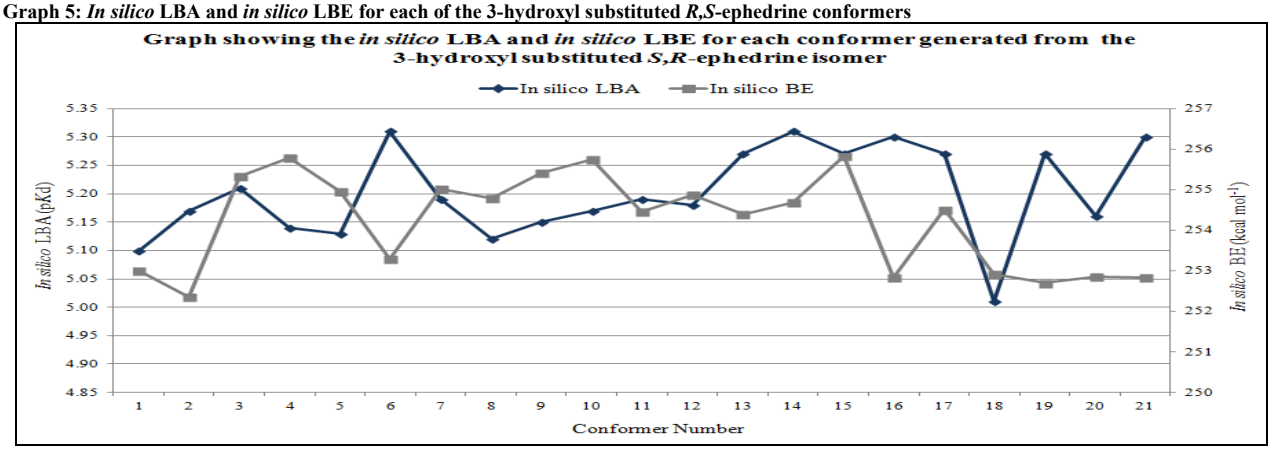
Graph 3: *In silico* LBA and *in silico* LBE for each of the *R,R*-ephedrine conformers



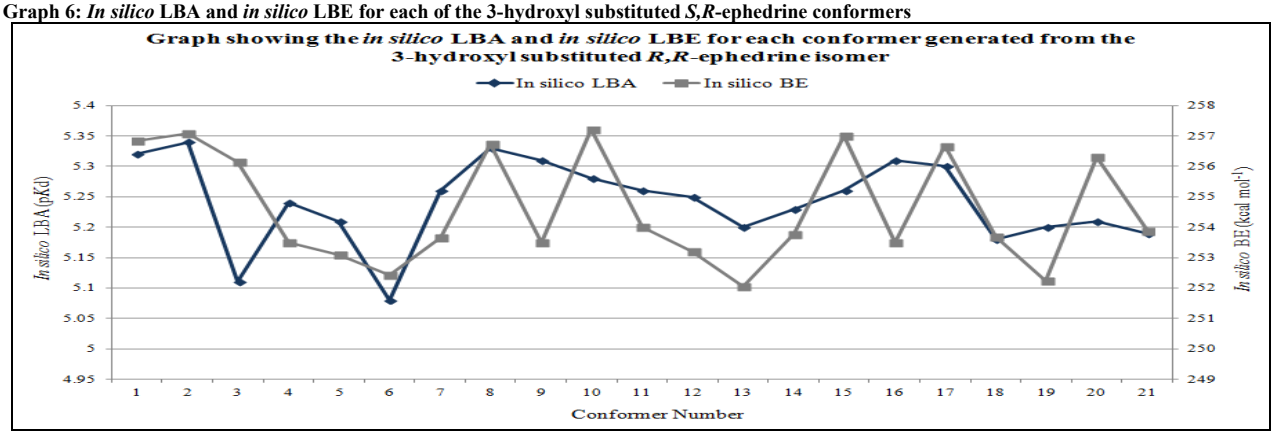
Graph 4: *In silico* LBA and *in silico* LBE for each of the *S,S*-ephedrine conformers



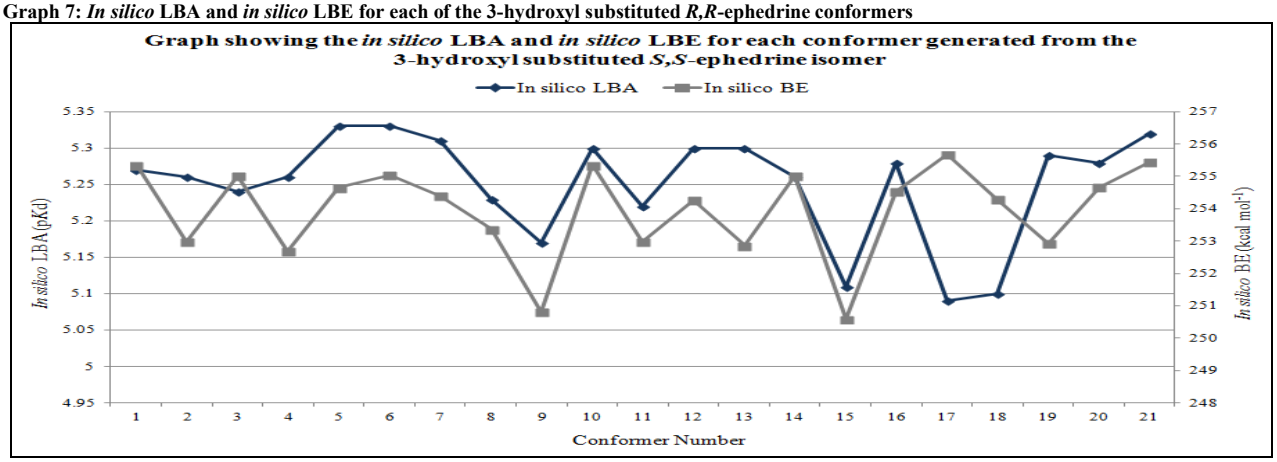
Graph 5: *In silico* LBA and *in silico* LBE for each of the 3-hydroxyl substituted *R,S*-ephedrine conformers



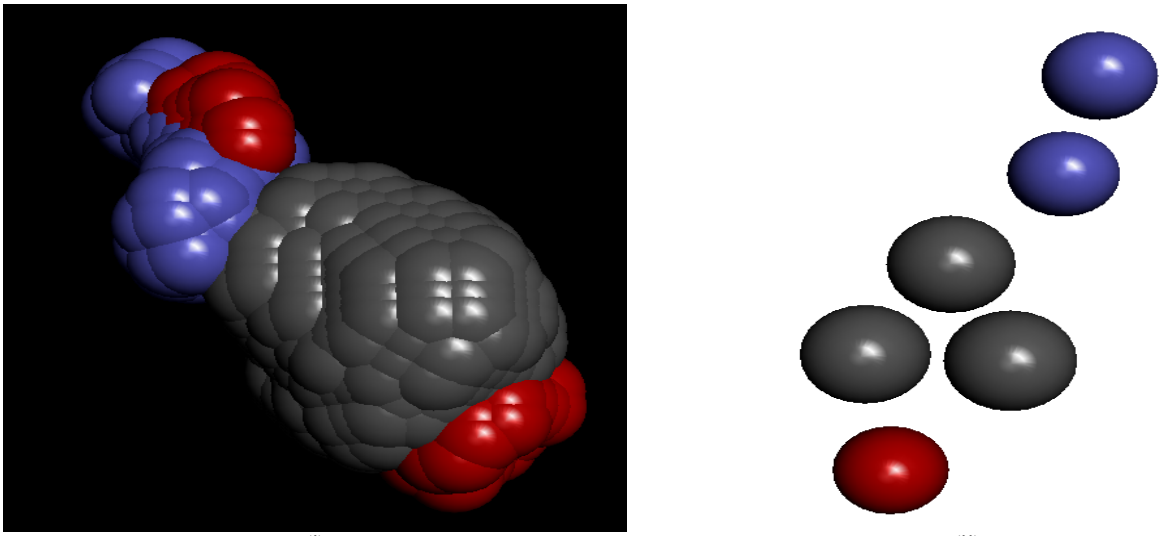
Graph 6: *In silico* LBA and *in silico* LBE for each of the 3-hydroxyl substituted *S,R*-ephedrine conformers



Graph 7: *In silico* LBA and *in silico* LBE for each of the 3-hydroxyl substituted *R,R*-ephedrine conformers



Graph 8: *In silico* LBA and *in silico* LBE for each of the 3-hydroxyl substituted *S,S*-ephedrine conformers



(i) (ii)

Fig. 5: The ligand binding pocket map generated in LigBuilder v.1.2 (i) describing the molecular volume occupied by 17-*β*-oestradiol within the interior of the ER and the corresponding proposed general pharmacophore (ii). Images rendered in Discovery Studio® v.3.5

Table I: Ligands with the highest LBA for the ER_LBP generated from each seed structure. Structural images were rendered in Sybyl®-X v.1.1

Seed	Chemical Formula	Molecular Wt.	Log P	p*K*d	2D structure
A	$C_{21}H_{17}N_2O_2$	329	4.45	8.55	
B	$C_{20}H_{18}NO_2$	304	4.93	8.02	
C	$C_{25}H_{21}NO_2$	367	4.82	9.99	
D	$C_{24}H_{27}N_2O_2$	375	4.72	8.35	

The *R,S*- diastereoisomer of ephedrine binds with higher affinity to the β-adrenergic receptors than do its isomeric cohorts [5] and was consequently used for the *de novo* phase of the study. Specifically the *R,S*- diastereoisomeric conformation from both the unsubstituted and the 3-hydroxyl substituted molecular cohorts exhibiting the highest LBA (p*K*d) and the lowest LBE (kcal mol^{-1}) (Fig.3) were selected as pharmacophoric platforms for the *de novo* phase of the study.

Two seed structures capable of sustaining molecular growth were created for each selected unsubstituted and 3-hydroxyl substituted conformer. This means that a total of four seed structures were considered in this study, two of which were derived from the unsubstituted *R,S*-conformation of ephedrine with the highest LBA (p*K*d) and lowest LBE (kcal mol^{-1}) and two of which were derived from the 3-hydroxyl substituted conformation with the highest LBA (p*K*d) and lowest LBE (kcal mol^{-1}).

RESULTS

The *in silico* LBA of the conformers generated from the *R,S*-diastereoisomer of ephedrine ranged from 5.02 to 5.24 where higher values indicate higher LBA. Conformer number 15, with a LBA (p*K*d) 5.24 and a LBE 245.81 kcal mol^{-1} was selected as a scaffold for the *de novo* design phase of the study on the basis of it possessing optimal attributes.

The *in silico* LBA of the conformers generated from the 3-hydroxyl *R,S*- diastereoisomer of ephedrine ranged from 5.01 to 5.33 where higher values indicate higher LBA. Conformer number 13, with a LBA (p*K*d) 5.28 and a LBE 252.08 kcal mol^{-1} was selected as a scaffold for the *de novo* design phase of the study on the basis of it possessing optimal attributes.

The *de novo* design process carried out in LigBuilder® v.1.2 generated a total of 755 molecules of which 233 were Lipinski rule compliant. The LBAs (p*K*d) of the molecules generated from seed A (n = 78) ranged from 7.00 to 8.55, those from seed B (n = 33) ranged from 7.14 to 8.02 while those for seed C (n = 87) ranged from 8.54 to 9.99. The LBAs (p*K*d) of seed D molecules (n = 24) ranged from 7.04 to 8.35.

DISCUSSION

The *in silico* docking of the four diastereoisomers of ephedrine and subsequent LBA (p*K*d) estimations did not corroborate Arbo *et al.*'s hypothesis of ER antagonism owing to the fact that their lower LBAs (p*K*d) indicate that it would be unlikely that 17-*β*-oestradiol would be displaced from the ER_LBP in their presence *in vivo*.

However, the fact that demonstrable albeit low affinity for the ER was recorded for all conformations provided the impetus for a *de novo* structure based drug design study that utilised the ephedrine scaffold as a novel base through which high affinity ligands capable of modulating the ER could be identified with the potential concomitant advantage of a reduced adverse effect profile.

Visual observation of the generated Lipinski rule compliant *de novo* generated ligands allowed a number of structural observations to be made. Specifically;

Increased bulk and the presence of two benzene rings linked together by a carbon atom, detracted from the LBA (p*K*d) of seed A derived molecules while the presence of amino moieties conversely, increased their affinity. In the case of seed B derived structures increased LBA was associated with the presence of a terminal amino group while a decrease in affinity was observed when this terminal amino group was replaced by a carbonyl moiety. Seed C derived ligands exhibited the highest affinity implying that this represented the optimal pharmacophoric scaffold for *de novo* design. The fact that seed C is 3-hydroxy substituted, augurs well for oestrogenic activity. Among this latter cohort the presence of a cyclopentene ring with allied carbonyl and amino moieties further increased the *in silico* estimated LBA. *De novo* designed ligands derived from seed D exhibited the lowest LBA. This may be explained structurally when it is taken into account that seed D lacks a hydrogen accepting oxygen atom attached to the benzyl moiety implying that a crucial hydrogen bond was disrupted at this locus (Fig. 4).

This study is significant in demonstrating that the hypothesis of Arbo *et al.* that ephedrine mediates an anti-oestrogenic effect does not hold through *in silico* calculations at the level of the ER. It remains however possible that ephedrine may still act as an anti-oestrogen through an alternative pathway further upstream from the ER.

The ephedrine scaffold proved of utility in the design of novel high affinity ligands for the ER, and the presence of a hydroxyl moiety at a locus analogous to the 3-hydroxyl group of 17-*β*-oestradiol augurs well for oestrogenic activity. The novelty of this molecular scaffold is interesting owing to the fact that the steroidal and Selective Oestrogen Receptor Modulators (SERMs) associated adverse effects are unlikely to be present. Ephedrine associated adverse effects would however have to be excluded in the context of further study.

The entire designed molecular cohort is consequently suitable for inclusion in molecular databases of utility for high throughput screening at the ER while the high affinity *de novo* ligands deriving from seed C may be suggested for further iterative rounds of optimisation and *in vivo* evaluation.

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