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Research article

THE DESIGN AND OPTIMISATION OF NOVEL STRUCTURES CAPABLE OF EPIDERMAL GROWTH FACTOR INHIBITION FOR THE MANAGEMENT OF NEOPLASTIC DISEASE

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ABSTRACT: Overexpression of Epidermal Growth Factor Receptors (EGFRs) due to gene amplification has been associated with the development of tumours of epithelial origin, including breast, lung, colon and ovarian. EGFRs are consequently targets for the design of antagonist molecules with the potential of solid tumour management. 2-O-caffeoyl tartaric acid, 2-O-feruloyl tartaric acid, Emetine and Rosmaricine are molecules for which there is evidence, from Chinese Pharmacopeia, of their ability to antagonise EGFR. These molecules were used as templates in the *de novo* design of novel EGFR inhibitors.

Protein databank crystallographic deposition 2ITY, describing the *holo*-gefitinib: EGFR complex, was used to define the pharmacophoric space available for novel molecular growth. 2-O-caffeoyl tartaric acid, 2-O-feruloyl tartaric acid, Emetine and Rosmaricine were successively docked into the EGFR ligand binding pocket (LBP) and conformational analysis performed. The optimal conformer for each molecule became the scaffold onto which novel moieties were computationally introduced at *loci* considered non-critical to binding using the GROW module of LigBuilder® v1.2.

66, 16, 17 and 55 molecules were designed from 2-O-caffeoyl tartaric acid, 2-O-feruloyl tartaric acid, Emetine and Rosmaricine scaffolds respectively after a larger cohort (*n*= 1770) was assessed for Lipinski Rule compliance. These molecules were classified according to pharmacophoric similarity, physiochemical parameters and ligand binding affinity. Their binding affinity (p*K*d), ranged between 10 and 5.76 compared to 6.05 for gefitinib.

The highest affinity Lipinski Rule compliant molecules are being suggested for further optimisation, synthesis and *in vitro* validation. This *in silico* study validated the utility of the selected lead scaffolds in the design of novel EGFR inhibitors**.**

Key words: EGFR, 2-O-caffeoyl tartaric acid, 2-O-feruloyl tartaric acid, Emetine, Rosmaricine

Abbreviations:

EGFR	Epidermal Growth Factor Receptor
LBA	Ligand Binding Affinity
LBE	Ligand Binding Energy
LBP	Ligand Binding Pocket
PDB	Protein Data Bank
TCM	Traditional Chinese Medicine

INTRODUCTION

Epidermal Growth Factor Receptors (EGFRs) are classified as receptor tyrosine kinases which are responsible for a number of cellular processes, such as: an increase in cellular proliferation, a decrease in apoptosis, enhanced tumour cell motility and neo-angiogenesis [1]. Therefore overexpression, due to gene amplification of the EGFR receptor, results in a number of tumours of an epithelial origin, such as: breast, lung, colon, ovarian and bladder [2].

This study aimed to assess the ability of the naturally occurring 2-O-caffeoyl tartaric acid, 2-O-feruloyl tartaric acid, Emetine and Rosmaricine, which were identified from Traditional Chinese Medicine (TCM), by Yang *et al*, (2011) [3] to antagonise the EGFR receptor. Based on the results obtained these molecules were used as templates in the *de novo* design of novel structures capable of inhibiting this receptor.

MATERIALS AND METHODS

The initial step of the study consisted of the identification and selection of a suitable 3D X-ray crystallographic structure of the target receptor, EGFR. The PDB crystallographic deposition 2ITY [4] was selected as a template for this study. The bound ligand, gefitinib, was extracted from the EGFR binding site and the *apo*-EGFR and the extracted ligand were saved in the PDB and mol2 formats respectively.

2D structures of each of the four ligands, identified from TCM, were sketched in Sybyl®-X v1.1 [5]. All of the four test ligands considered in this study were chiral. The literature reviewed specified which stereoisomer is bioactive for all ligands, except for 2-O-caffeoyl tartaric acid. Consequently in this latter case, all possible chiral combinations were sketched. 2-O-caffeoyl tartaric acid had two chiral centres therefore a total of four stereoisomeric combinations were constructed. Once the 2D structures were drawn, the molecules were optimised and saved in a mol2 file. Figure 1 represents the 3D structures of the sketched test ligands rendered in Chimera® v1.7 [6]

As none of the 3D structures of the ligands were resident within the original PDB file conformational analysis was carried out using Sybyl®-X v1.1 [5]. The bound co-ordinates of gefitinib were used as templates and twenty possible conformations were obtained for each ligand. Each conformation was then saved as a separate mol2 file and this process was carried out for all the ligands.

The PDB file, containing the co-ordinates for the *apo*-EGFR receptor, and all the mol2 files, for all the possible conformations, were exported to the Unix-based programme XSCORE v1.3 [7]. XSCORE v1.3 [7] was then utilised to calculate the Ligand Binding Affinity (LBA) for all the possible conformations for all the ligands at the EGFR binding site. The Ligand Binding Energy (LBE) (Kcal mol^{-1}) was also computed for each individual conformation using Sybyl®-X v1.1 [5].The best conformer for each test ligand was identified by using the combination of the highest LBA (p*K*d) and lowest LBE (Kcal mol^{-1}) as selection criteria.

The selected conformers were used in the creation of seed molecules using Sybyl®-X v1.1 [5]. Modifications to the structure were made based on the known structure-activity relationship as discussed by Yang *et al* (2011) [3]. The growing sites on the seed scaffolds were labelled using the *H.spc* atom.

The binding pocket was analysed in LigBuilder® v1.2 [8]. Thus the pharmacophoric structure and key interactions were elucidated. The GROW function was utilised to construct ligands for the target binding site from the seed structures created.The final module, PROCESS, was utilised to generate mol2 files for the novel structures created in the GROW module and organise them into families, based on their structural similarities.

The next step of the study consisted in the analysis of the results. Novel molecules were identified and selected on the basis of their drug-like qualities by using Lipinski's Rule of Five [9][10]as a guideline. Once this was done the high affinity and low affinity structures of molecules residing within the same family were compared, followed by structural comparison of the highest rankers from each family. The substituents essential to binding to the EGFR_LBP were then identified.

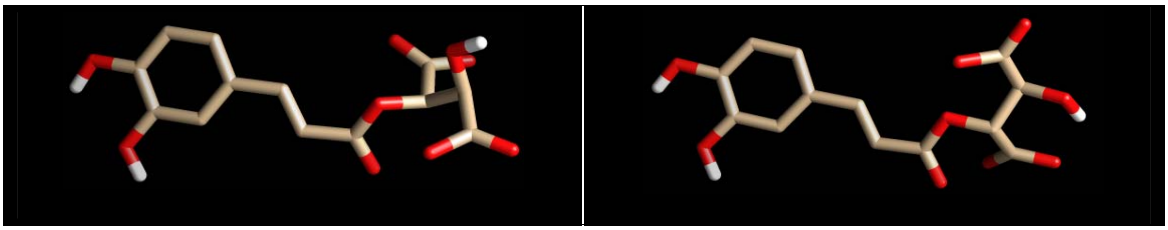
RESULTS

To identify the best conformers for each test ligands the LBA (p*K*d) and the LBE (Kcal mol^{-1}) were plotted together for each conformation for each test ligand. Four graphs were plotted for 2-O-caffeoyl tartaric acid. Each graph represented the different LBAs (p*K*d) and LBE (Kcal mol^{-1}) obtained for each chiral possibility (R,R; R,S; S,R and S,S) (Refer to Figures2 – 5). The best conformation from each graph was selected and another graph was then plotted using the selected conformations to identify the best one (Refer to Figure 6). The remaining graphs for 2-O-feruloyl tartaric acid, Emetine and Rosmaricine were also plotted (Refer to Figures 7 – 9). For 2-O-caffeoyl tartaric acid, conformation 2, which was obtained from the lead molecule with chiral centres R,R, was selected. Conformation 11, 12 and 1 were selected for 2-O-feruloyl tartaric acid, Emetine and Rosmaricine respectively.

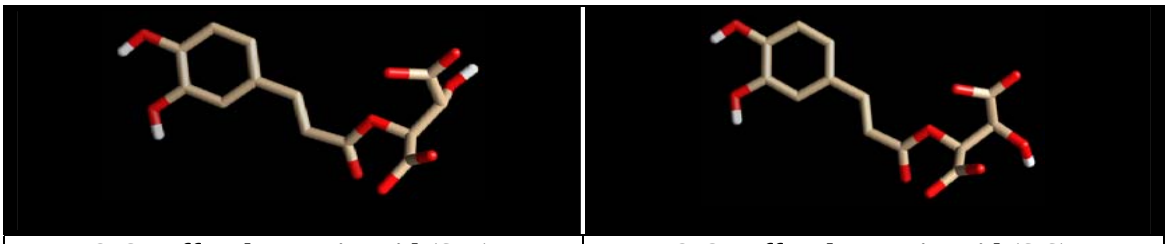
Four seed molecules were created for 2-O-caffeoyl tartaric acid and Emetine, two seeds were created for 2-O-feruloyl tartaric acid and three seed molecules were created for Rosmaricine. Their 2D structures, which were rendered Accelrys Draw [11], can be seen in Table 1.

The GROW and PROCESS modules generated a total of 1770 novel molecules from the seed structures,of which 154 were found to be Lipinski Rule compliant (66 novel molecules were generated from 2-O-caffeoyl tartaric acid, 16 from 2-O-feruloyl tartaric acid, 17 from Emetine and 55 from Rosmaricine).

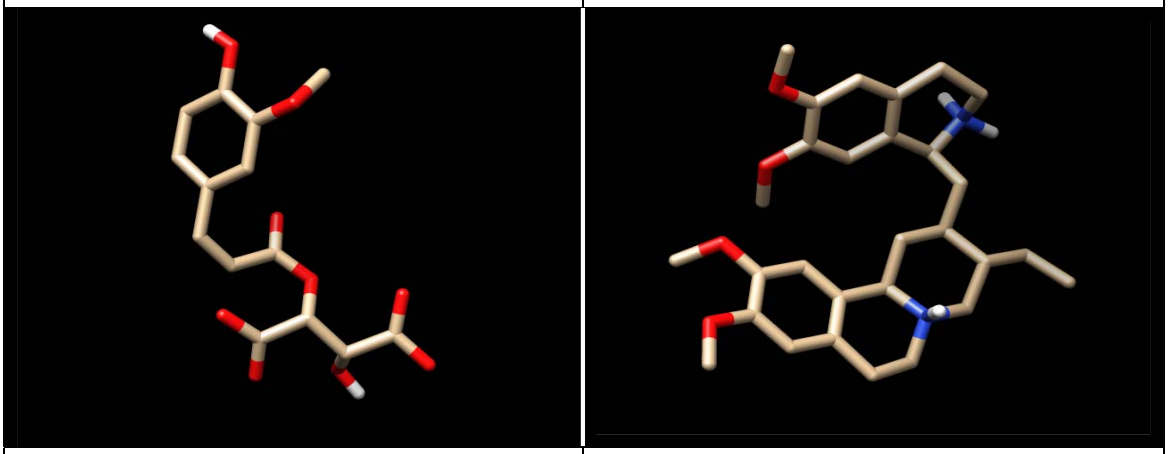
Analysis of the novel structures resulted in identification of the moieties essential for binding of the molecules to the EGFR_LBP (Refer to Table 2). Furthermore the substituents were linked to propose a total of four ideal molecules. (Refer to Figures10 – 14).



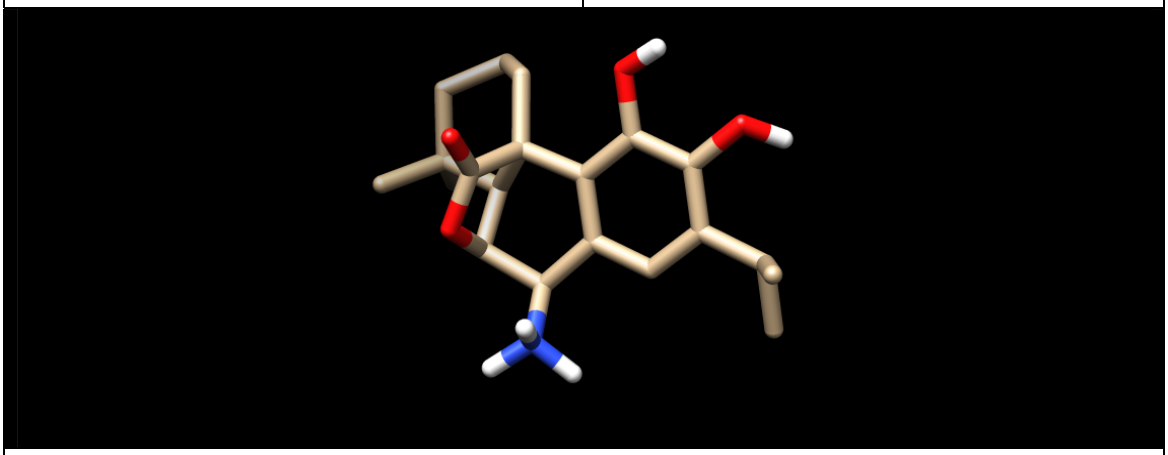
2-O-caffeoyl tartaric acid (R,R) **2-O-caffeoyl tartaric acid (R,S)**



2-O-caffeoyl tartaric acid (S,R) **2-O-caffeoyl tartaric acid (S,S)**



2-O-feruloyl tartaric acid **Emetine**



Rosmaricine

Figure 1: 3D structures of the sketched test ligands. Images were rendered in Chimera(R) v1.7 [6]

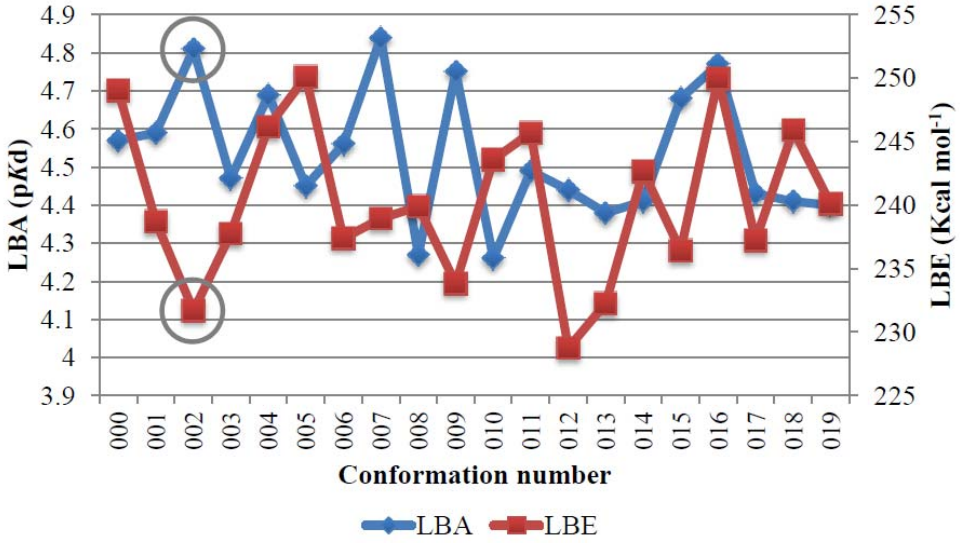


Figure 2: Graph showing the LBA (p*K*d) and LBE (Kcal mol^{-1}) for each of the 20 conformers for 2-O-caffeoyl tartaric acid (R,R) within the EGFR_LBP

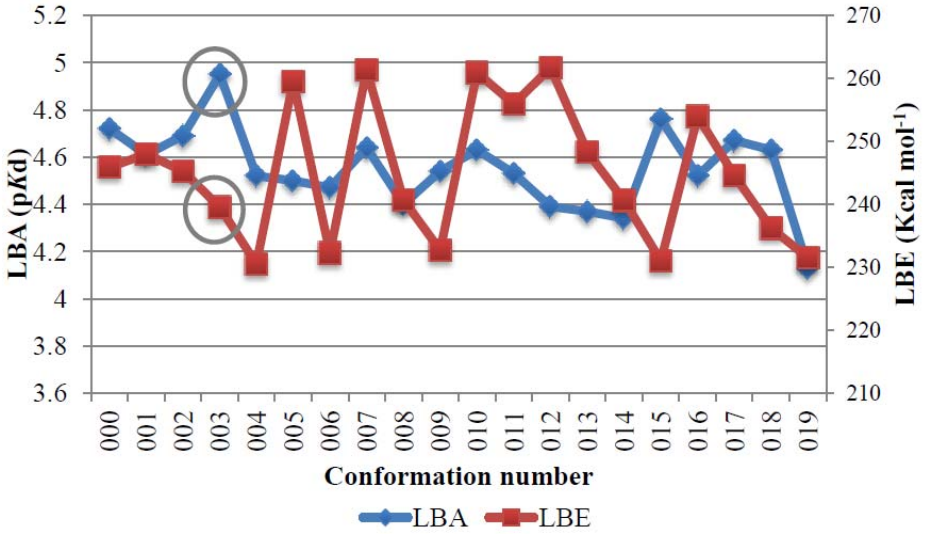


Figure 3: Graph showing the LBA (p*K*d) and LBE (Kcal mol^{-1}) for each of the 20 conformers for 2-O-caffeoyl tartaric acid (R,S) within the EGFR_LBP

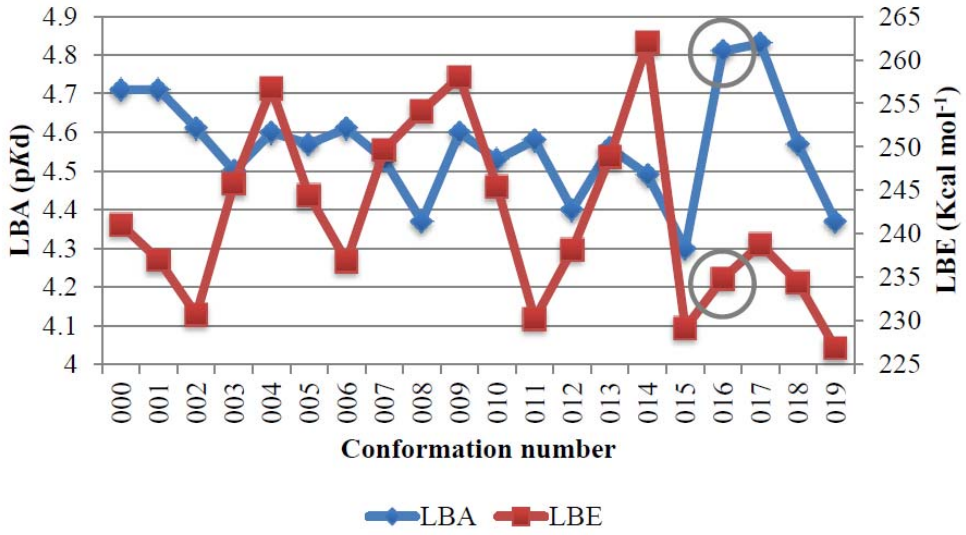


Figure 4: Graph showing the LBA (p*K*d) and LBE (Kcal mol^{-1}) for e ach of the 20 conformers for 2-O-caffeoyl tartaric acid (S,R) within the EGFR_LBP

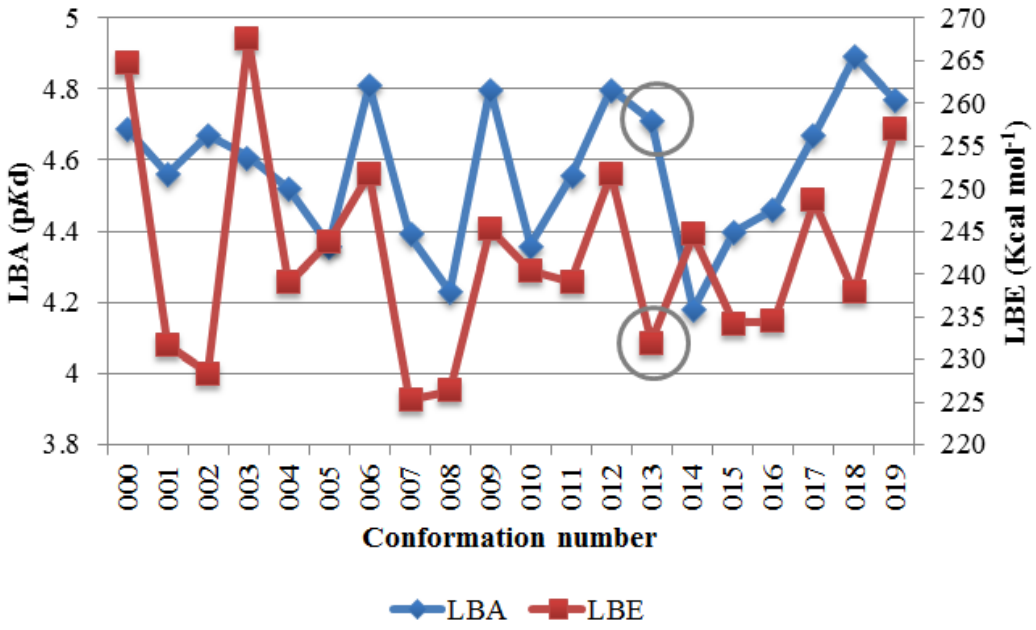


Figure 5: Graph showing the LBA (p*K*d) and LBE (Kcal mol^{-1}) for each of the 20 conformers for 2-O-caffeoyl tartaric acid (S,S) within the EGFR_LBP

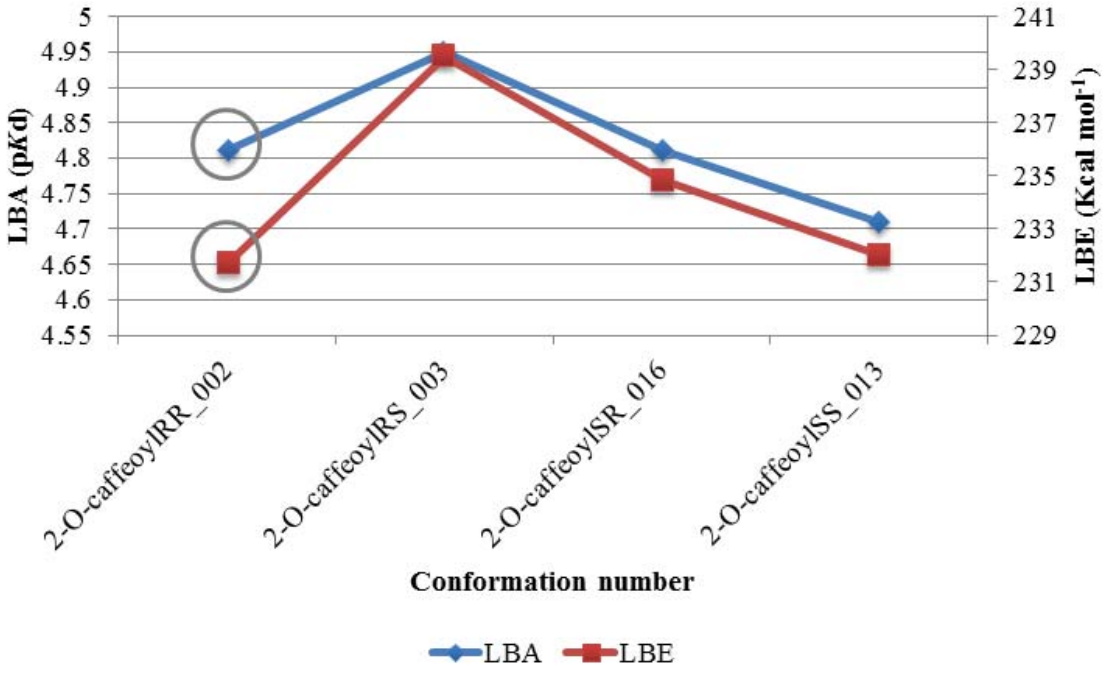


Figure 6: Graph showing the LBA (p*K*d) and LBE (Kcal mol^{-1}) for each of the best conformers selected for 2-O-caffeoyl tartaric acid within the EGFR_LBP

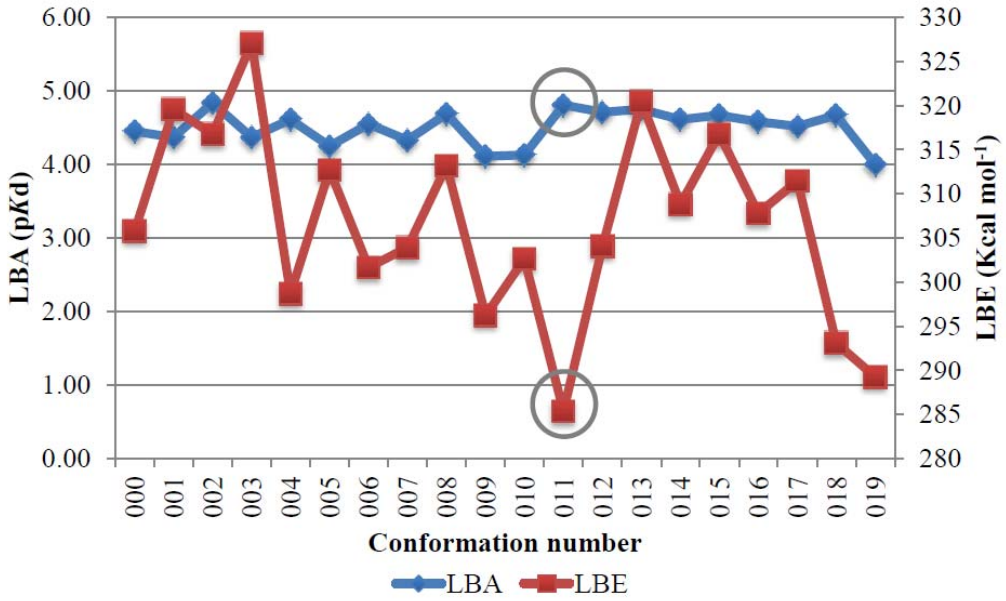


Figure 7: Graph showing the LBA (p*K*d) and LBE (Kcal mol^{-1}) for each of the 20 conformers for 2-O-feruloyl tartaric acid within the EGFR_LBP

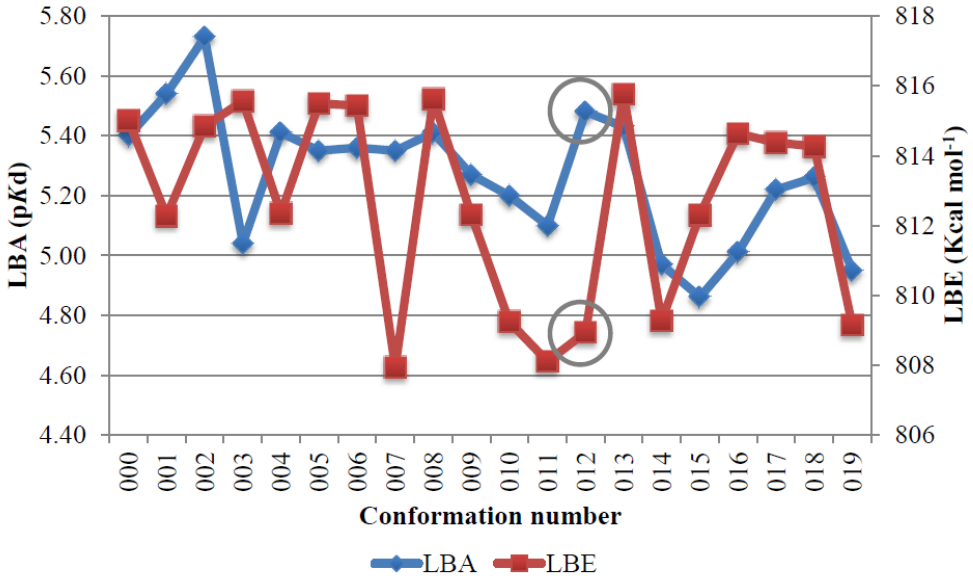


Figure 8: Graph showing the LBA (p*K*d) and LBE (Kcal mol^{-1}) obtained for each of the 20 conformers for Emetine within the EGFR_LBP

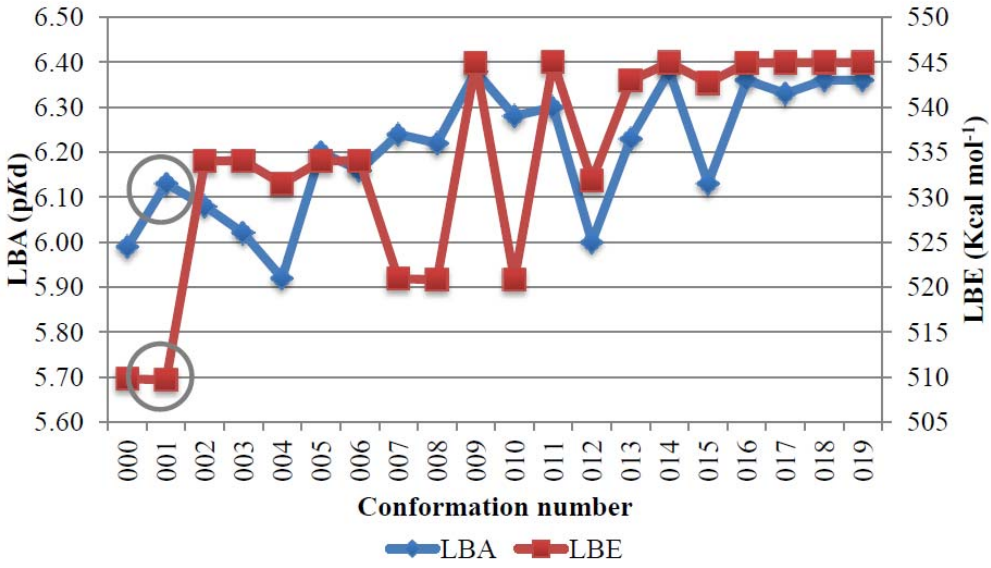


Figure 9:Graph showing the LBA (p*K*d) and LBE (Kcal mol^{-1}) obtained for each of the 20 conformers for Rosmaricine within the EGFR_LBP

Table 1: 3D structures of the seed molecules created from 2-O-caffeoyl tartaric acid, 2-O-feruloyl tartaric acid, Emetine and Rosmaricine. Images were rendered in Chimera® v1.7 [6]

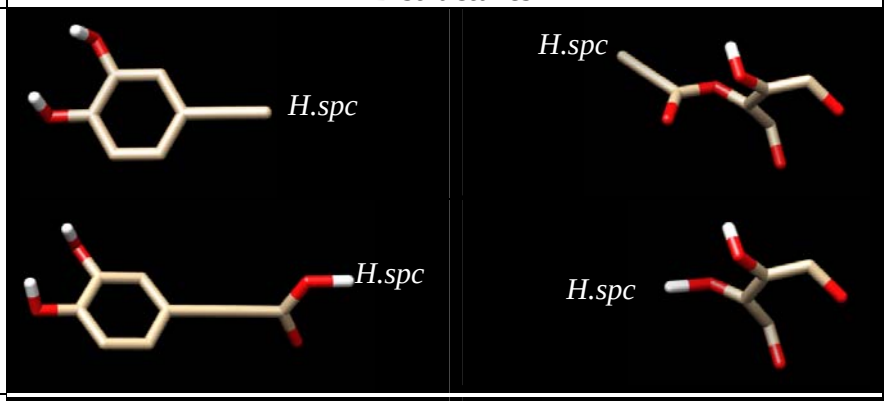
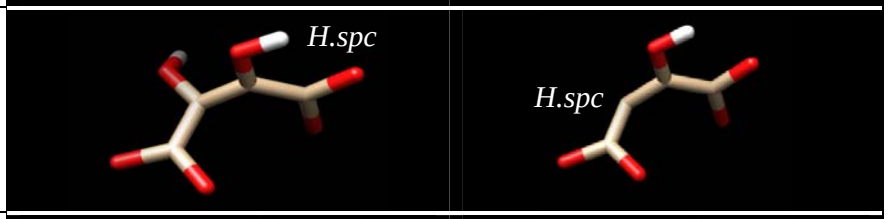
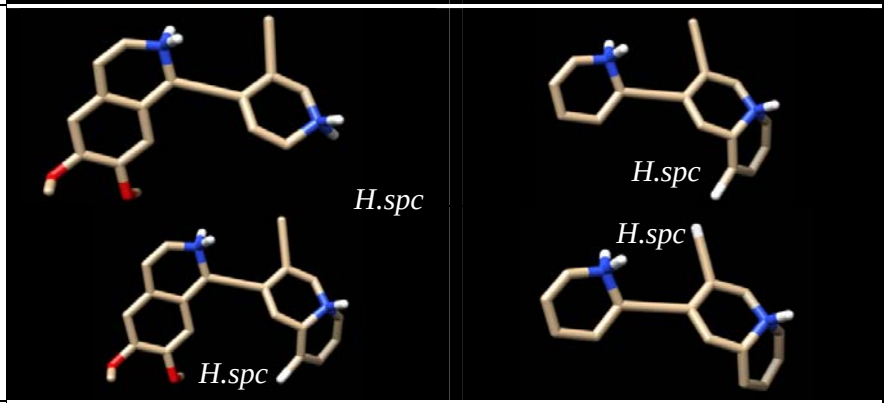
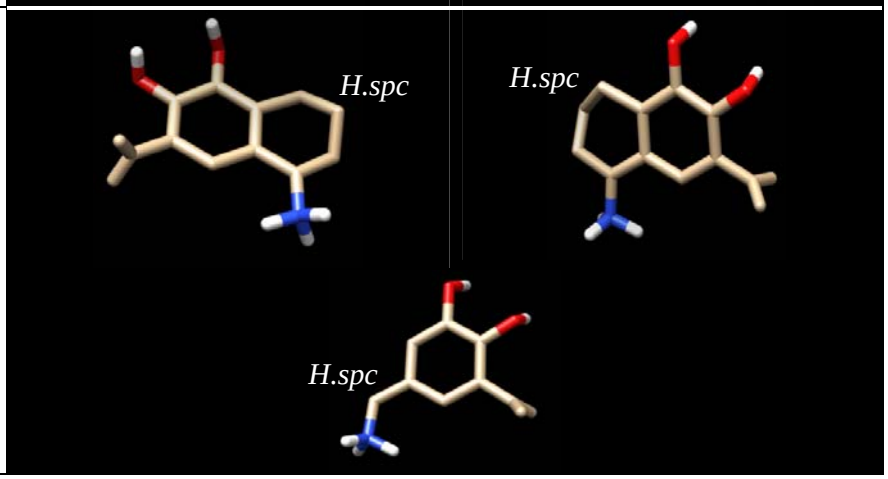
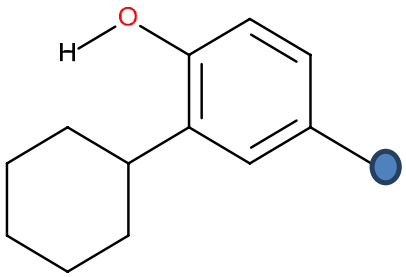
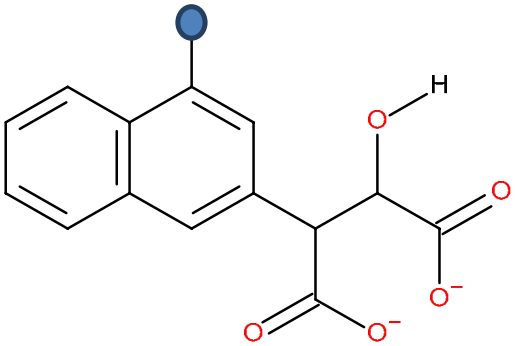
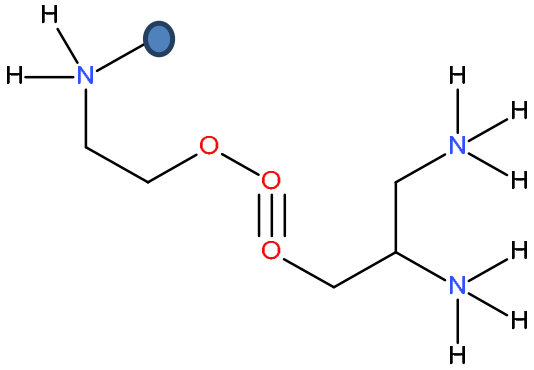
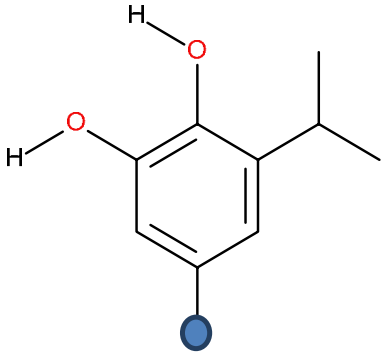
Test ligand	2D structures
2-O-caffeoyl tartaric acid	
2-O-feruloyl tartaric acid	
Emetine	
Rosmaricine	

Table 2: Moieties found to be essential for good binding affinity. Images were rendered in Accelrys Draw® 4.1 [11]

Original molecule	2D representation of the moieties essential for binding
2-O-caffeoyl tartaric acid	
2-O-ferulyol tartaric acid	
Emetine	
Rosmaricine	

- Symbol depicts the anchor site

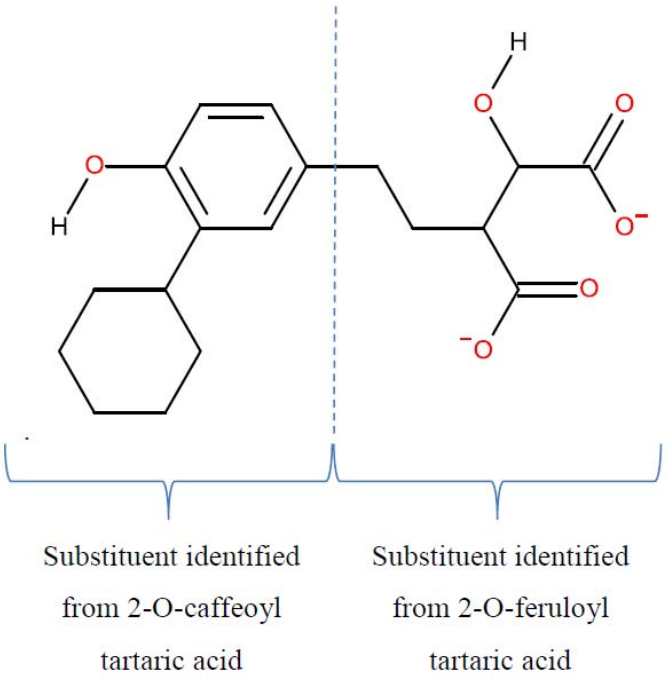


Figure 10: 2D structure showing a recommended ideal structure (1). Images were rendered in Accelrys Draw® 4.1 [11]

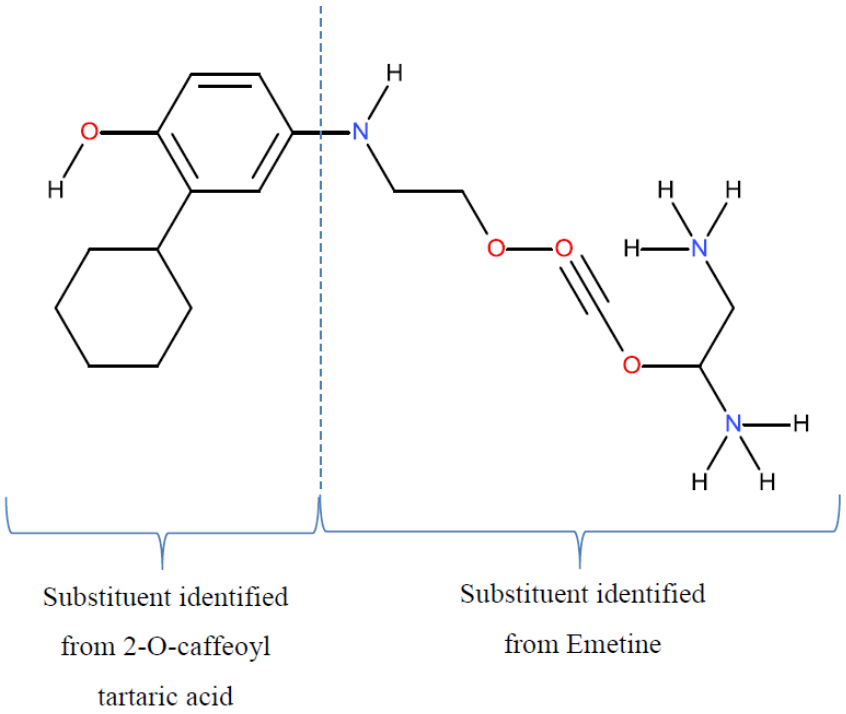


Figure 11: 2D structure showing a recommended ideal structure (2). Images were rendered in Accelrys Draw® 4.1 [11]

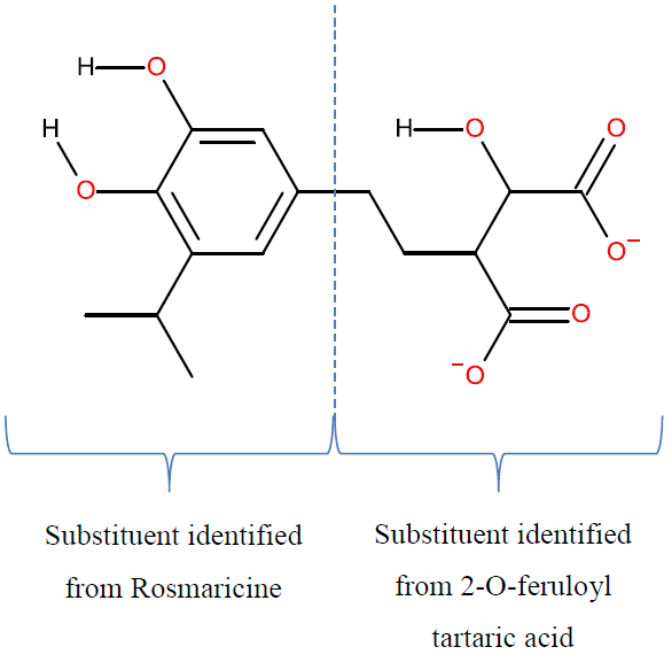


Figure 12: 2D structure showing a recommended ideal structure (3). Images were rendered in Accelrys Draw® 4.1 [11]

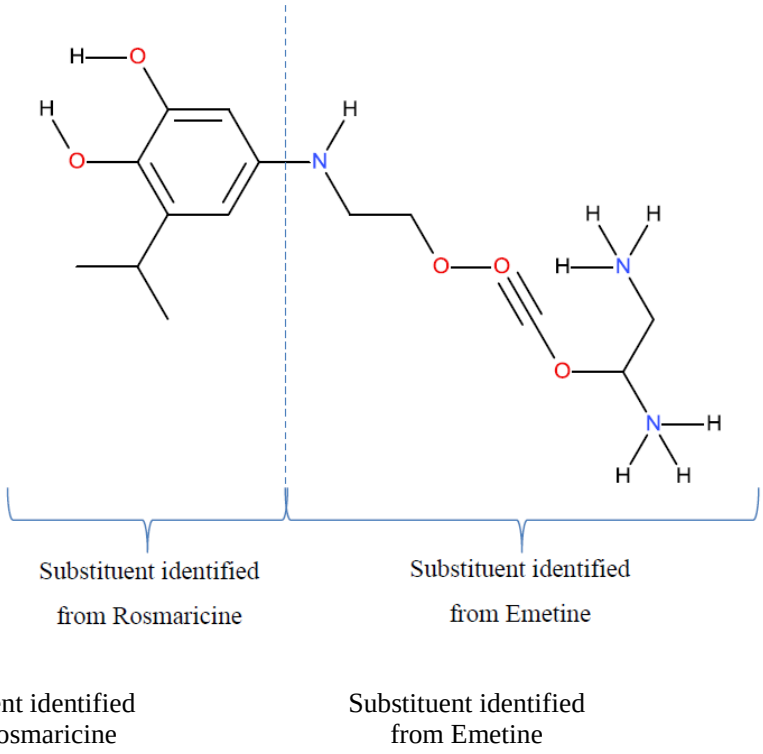


Figure 13: 2D structure showing a recommended ideal structure (4). Images were rendered in Accelrys Draw® 4.1 [11]

Table 3: LBA (p*K*d) of Gefitinib and that of the best conformation for each test ligand

Molecule	LBA (p*K*d)
Gefitinib	6.05
2-O-caffeoyl tartaric acid	4.81
2-O-feruloyl tartaric acid	4.81
Emetine	5.48
Rosmaricine	6.13

DISCUSSION

The initial phase of the study aimed to establish the LBA (p*K*d) of the four test ligands for the EGFR receptor and comparing this to the affinity of the established ligand gefitinib. Gefitinib was selected as a reference for this study owing to the fact that it is an effective EGFR tyrosine kinase inhibitor, currently being used in cancer treatment. It was also the reference molecule used by Yang *et al* (2011) [3] when identifying candidates from TCM for their potential EGFR inhibitory effect. The LBA (p*K*d) of three of the four test ligands was found to be lower than that of gefitinib however these lead molecules still have the potential of being good scaffolds in the creation of novel molecules. (Refer to Table 3)

The LBA (p*K*d) of the novel molecules, generated from the seed structures, found to be Lipinski Rule complaint [9][10] was then determined. From the 154 Lipinski Rule compliant molecules generated only eleven were found to have a lower LBA (p*K*d) when compared to gefitinib (p*K*d = 6.05).

The substituents identified to be essential in the binding of novel molecules to the EGFR_LBP were linked, and four ideal molecules were proposed. By combining these moieties more points of attachment for the ligand to the EGFR would be created, thus making the ligands more specific to their target and therefore achieve a reduced side-effect profile.

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