Research Article

Synthesis of Precursors of Antiosteoporotic Agents and its QSAR Studies on EGFR Receptor Inhibitors

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Abstract

The National Institutes of Health defines osteoporosis as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. However, currently there is no definition that is agreeable to both medical and scientific communities and its etiology is poorly understood. It is within this framework that the pharmaceutical industry is trying to develop new treatments for the so-called silent epidemic. This research article describes the osteoporosis as a disease and look forward for the update in its management with an aim to synthesize precursors of antiosteoporotic agents and QSAR studies on EGFR receptor inhibitors.

Introduction

There have been many works published about the design, conduct, and analysis. Why osteoporosis trials are special case that deserves a work of their own? There are three main reasons. First, most diseases have a well-understood definition and etiology. Osteoporosis is a disease that is understood by those working within the subspecialty, but currently there is no definition that is agreeable to both medical and scientific communities and its etiology is poorly understood. It is within this framework that the pharmaceutical industry is trying to develop new treatments for the so-called silent epidemic. In layman’s terms, the disease of osteoporosis is defined as brittle bones occurring in the elderly that could lead to fractures. The classical definition was “a bony fracture caused by minimal trauma owing to a loss in bone mineral”. A published consensus definition states that osteoporosis is “a systemic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures”. The National Institutes of Health (NIH) Consensus Conference Statement on Osteoporosis Prevention, Diagnosis, and Therapy states that “osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture”. The World Health Organization (WHO) operationally defines osteoporosis as “bone density 2.5 standard deviations (SDs) below the mean for young white adult women at lumbar spine, femoral neck, or forearm”. It is now recommended that the diagnostic use of this definition is restricted to bone density of the femur. Although it is not clear how to apply this in men and children, it is recommended that the same diagnostic thresholds can be used in men. The NIH statement recognizes that bone strength reflects the integration of two main features: bone density and bone quality. Currently, there is no accurate measure of overall bone strength. Bone mineral density (BMD) is frequently used as a proxy measure and accounts for approximately 70% of bone strength. Thus, osteoporosis has become a disease that is characterized by measurement of BMD. The endpoint of many clinical trials is BMD, either used as a primary endpoint in its own right or used as a surrogate marker for fracture risk. Regulatory authorities tend to consider osteoporosis in terms of fracture when it comes to licensing new treatments.
for the management of the disease, and increasingly, BMD for the prevention of osteoporosis. It is, therefore, imperative that the researcher understands which definition of the disease they are using and what the endpoint or hypothesis they are trying to evaluate is before they embark on a research program.

Second, because osteoporosis is a disease that is diagnosed using a measurement of BMD and is monitored over many years using such measurements, there are a range of technical issues to ensure the quality and consistency of BMD measurements that must be considered. Several of these relate to the choice of equipment, standardization, and quality control before a trial begins, in addition to technical issues that must be considered throughout the life of the study.

Third, osteoporosis trials are often long-term trials carried out in normal, asymptomatic women, in whom proven drugs for the treatment and prevention of osteoporosis are already licensed. This is particularly true of clinical trials in women who are close to the menopause. This presents ethical issues because the latest version of the Declaration of Helsinki (Finland), produced in Edinburgh (UK) in 2000, specifically states that placebo control in the presence of a proven treatment is unethical. This conflicts with the requirements of the US Food and Drug Administration (FDA), which still requires placebo control for licensing purposes. These women are also unlikely to gain any direct benefit from a short-term trial, which raises other ethical issues. Postmenopausal women (aged 55 to 65 years) are unlikely to have any long-term reduction in fracture risk if the fracture does not occur until they are aged 80 years. Any protective effect of treatment will have worn off. What happens at the end of the study? Will treatment still be available to subjects if a proven treatment effect is demonstrated? In summary, the definition of osteoporosis is not universally agreed, it is a disease defined by a measurement of BMD and often clinical trials are carried out in normal, asymptomatic women. For researchers entering into this therapeutic area, it seems to be initially confusing and technically challenging. On this basis, osteoporosis clinical trials deserve a work that provides an introduction to the novice and clearly explains the design and implementation of these trials.

Therapies for osteoporosis are licensed for either prevention or treatment, or both this distinction is somewhat artificial and whether a treatment is used in either way will tend to depend more on the balance between risks and benefits and whether the treatment is acceptable to the subject and cost effective. Osteoporosis itself is asymptomatic and its clinical significance is that it is an important modifiable risk factor for low-trauma fracture. When selecting a therapy, it is more relevant that the treatment has anti-fracture efficacy and to determine whether this efficacy is for both vertebral and nonvertebral fractures. Other desirable characteristics of a pharmacological intervention for osteoporosis are safety and tolerability. Ideally, a preparation should be easy to take because this will improve the chances of both compliance and persistence with treatment. Cost-effectiveness is increasingly determining which preparations healthcare organizations will permit clinicians to prescribe and in England; the National Institute for Health and Clinical Effectiveness (NICE) and the activities of prescribing advisers and formulary committees are very influential in this process. It must be remembered that the majority of the RCTs that have been published are in Caucasian postmenopausal women. There are substantial variations in the prevalence of osteoporosis and osteoporotic fractures in different countries, even in this group of subjects. Although probably equally effective in men, the evidence base is limited. We have little knowledge of the efficacy of treatments in racial groups other than Caucasians, in which the absolute fracture risk could be much lower and, therefore, cost-effectiveness more difficult to demonstrate. Treatments for osteoporosis aimed at reducing fractures can broadly be divided into three groups: those that reduce resorption by inhibiting osteoclastic activity, those that have anabolic functions that stimulate osteoblastic activity to lay down more bone, and one preparation that seems to have a dual action. The aim of this study is to synthesize precursors of antosteoporotic agents and QSAR studies on EGFR receptor inhibitors.

**Experimental**

**Materials and Equipments:** All chemicals used were of reagent grade. All the solvents used for the reactions and purifications were commercially available and used after distillation. Melting points were determined on a complab melting point apparatus and are otherwise uncorrected. Reactions were monitored by thin layer chromatography on self made plates of silica gel-G (Merck, India; Qualigens) or 0.25 mm readymade plates of silica gel and the detection was done by iodine vapors, spraying with Dragendorff's spray reagent, ninhydrin reagent, potassium permanganate spray or by UV radiation. Compounds were purified by column chromatography performed with glass columns using silica gel (60-120 mesh; Merck, Qualigens), as stationary phase and solvent/mixture of solvents as mobile phase or Chromatotron apparatus (Made in USA). The infrared spectra (λ max in cm⁻¹) were recorded with Perkin Elmer 881 spectrophotometer in KBr or Neat. 1H NMR spectra (ppm, δ) were recorded at 200MHz on Bruker AVANCE DPX 200 and 300MHz on Bruker AVANCE DPX 300 with tetramethylsilane (TMS) as the internal standard. Coupling constants (J) are reported in Hertz (Hz), and s, d, t, m and bs refer to singlet, doublet, triplet, multiplet and broad respectively. FAB mass spectra
were recorded on JEOL SX 102/DA 6000 mass using Argon /Xenon (60 KV, 10 MA) as the FAB gas or ESI-MS were recorded on Quatro II spectrometer. Elemental analyses were carried out on Carlo ERBA CHNS-OEA1108-Elemental Analyzer.

**Synthesis of 2-(benzylamino)-1-phenylethanol:** In a dry round bottom flask 11 g of benzyl amine was taken along with 12.36 g of styrene oxide under nitrogen atmosphere and added SiO₂ by 10% w of the initial, allowed the reaction mixture to stir over night under nitrogen atmosphere. After completion of the reaction (as per the TLC in the solvent system 9:1 DCM: hexane, R\textsubscript{s} = 0.65). 200ml of diethyl ether was added and 2-3 drops of water for settling the catalyst. Then filtered the solvent under suction and allowed the reaction mixture to be crystallized. The crystallized product was filtered, dried and weighed 10% at. SiO₂ N₂ atm.

![Scheme 1: Functional group transformation for alcoholic group.](image)

**Formation of N-benzyl-2-bromo-2-phenylethanamine:** 500mg of 2-(benzyl amino)-1-phenylethanol was dissolved in dry DCM/dry THF (10ml+5ml) then added 0.8ml of phosphorus tribromide dissolved in 10ml of dry DCM dropwise at -20°C with the help of dropping funnel salt formed with the amine part get separated which on further addition get dissolved and the reaction was stirred for 45 min then another 0.4ml of phosphorus tribromide dissolved in 1ml of DCM was added and allowed the reaction to stir for 1hr at the same temperature. TLC was operated (2.5/ EtOOAc:Hex). After the completion of the reaction the excess of phosphorus tribromide was removed under pressure in an efficient hood. Then added aqueous ammonia at such a rate that the temperature should not increase more then -5°C then extracted with ethylacetate (2×50ml) dried the organic layer over anhyd. Na\textsubscript{2}SO\textsubscript{4} concentrated to obtain the desired product.

![Formation of 3-(benzylamino)-2-phenylpropanenitrile](image)

**O-acetylation of 2-(benzylamino)-1-phenylethanol:** Half mg of the Boc protected compound was dissolved in 15 ml of pyridine (30-40 eq.) then cooled the mixture to -10°C and slowly added Acetic anhydride 2 ml (5-6 eq.) with vigorous stirring at such rate that the temperature should be maintained. After 5 h the reaction mixture was concentrated in high vacuum added 60 ml of chilled water and extracted with ethylacetate (3×100ml) dried the organic layer over Na\textsubscript{2}SO\textsubscript{4} concentrated in vacuo to obtain Boc protected product. Deprotection was carried out using 5 ml TFA: DCM (1:1) after completion of the deprotection the reaction mixture was concentrated and then washed with water extracted with DCM drying the organic layer over sodium sulfate and concentrated under vacuo.

**O-benzoylation of 2-(benzylamino)-1-phenylethanol:** One hundred and sixty four mg of NaH (2.5 eq. washed with hexane to remove the mineral oil) was taken in 50 ml flask added 500 mg of the Boc protected compound dissolved in 15 ml of dry DCM was added at -10 °C and slowly added benzoyl
chloride 0.5ml (1.5-2eq.) with vigorous stirring and allowed the reaction mixture to achieve room temperature. After 2.5 h the reaction mixture was concentrated in high vacuum added 60 ml of chilled water and extracted with ethylacetate (3×100 ml) dried the organic layer over Na2SO4 concentrated in vacuo to obtain viscous product. Deprotection was carried out using 5ml TFA:DCM (1:1) after completion of the deprotection the reaction mixture was concentrated and then washed with water extracted with DCM drying the organic layer over sodium sulfate and concentrated under vacuo.

0-benzylation of 2-(benzylamino)-1-phenylethanol: One hundred and sixty four mg of NaH (2.5 eq. washed with hexane to remove the mineral oil) was taken in 50 ml flask added 500 mg of the Boc protected compound dissolved in 15 ml of dry DCM was added at -10 °C and slowly added benzyl chloride 0.5 ml (3 eq.) with vigorous stirring and allowed the reaction mixture to achieve room temperature. After 2.5 h the reaction mixture was concentrated in high vacuum added 60 ml of chilled water and extracted with ethylacetate (3×100 ml) dried the organic layer over Na2SO4 concentrated in vacuo to obtain viscous product. Deprotection was carried out using 5 ml TFA:DCM (1:1) after completion of the deprotection the reaction mixture was concentrated and then washed with water extracted with DCM drying the organic layer over sodium sulfate and concentrated under vacuo.

O-tosylation of 2-(benzylamino)-1-phenylethanol: One hundred and thirty four mg of NaH (1.8 eq. washed with hexane to remove the mineral oil) was taken in 50 ml flask added 500 mg of the Boc protected compound dissolved in 15 ml of dry DCM was added at -10 °C and added tosyl chloride 0.4 g (1.5 eq.) with vigorous stirring and allowed the reaction mixture to achieve room temperature. After 4 h the reaction mixture was concentrated in high vacuum added 60 ml of chilled water and extracted with ethylacetate (3×100 ml) dried the organic layer over Na2SO4 concentrated in vacuo to obtain viscous product. Deprotection was carried out using 5ml TFA:DCM (1:1) after completion of the deprotection the reaction mixture was concentrated and then washed with water extracted with DCM drying the organic layer over sodium sulfate and concentrated under vacuo.

Synthesis of 3-benzyl-2,2-dimethyl-5-phenyloxazolidine: Five hundred mg of 2-(benzylamino)-1-phenylethanol was dissolved in dry acetone (10 ml) then added 5 ml of 2,2-dimethoxypropane at 0 °C and added 2-3 drops of boron trifluoride and stirred the reaction for 45 min. After completion of the reaction as suggested by the TLC (1:3/acetone:hexane) the reaction mixture was concentrated under vacuo at a temperature of 40 °C. The solid separated was filtered dried and weighed.
Synthesis of 2-(3-benzyl-4-methoxy-4-oxobutanoyl) benzoic acid: Five g of 2-(3-benzyl-4-methoxy-4-oxobutanoyl) benzoic acid was taken in 250 ml flask charged with dry DCM (100 ml) and cooled at 0 °C and added 8.1 ml TEA (2.4 eq) after 15 min of stirring. 4.1 g of phthalic anhydride (1.3 eq) was added to the reaction mixture. Then, allowed the reaction to stir overnight. After the completion of the reaction 100 ml of 1N HCl solution was added and the solid lithium salt was separated by filtration and then added 20 ml of 1N HCl solution to neutralize the salt separated then extracted with ethylacetate (60 ml×3), dried over anhyd. Na2SO4 and concentrated in vacuo.

Preparation of tryptophan methyl ester hydrochloride: Ten mg of tryptophan was taken in a 1000 ml flask and added 750 ml absolute methanol and brought the temperature of the suspension thus obtained to 0-5 °C. Then added (5ml+1ml) thionyl chloride to the reaction mixture and allowed to attain the room temperature then stirred for 10-14 h. On completion of the reaction as per the TLC product was filtered under suction.

Synthesis of 2-(3-(1H-indol-3-yl)-1-methoxy-1-oxopropan-2-ylcarbamoyl) benzoic acid: Five g of tryptophan methyl ester was taken in 250 ml flask charged with dry DCM (100 ml) and cooled at 0 °C and added 8.1 ml TEA (2.4 eq) after 15 min of stirring. 4.1 g of phthalic anhydride (1.3 eq) was added to the reaction mixture. Then, allowed the reaction to stir overnight. After the completion of the reaction 100 ml of 1N HCl solution was added and organic layer was separated and washed with water (60 ml×3) and brine (60 ml×2) and dried over Na2SO4 then concentrated in vacuo.

Result and Discussion

Synthesis of 2-(benzylamino)-1-phenylethanol: Yield: 19.3 g (88%), Melting point: 234-237 °C, IR (cm⁻¹): 3317.5, 3052.0, 2925.3, 1588.8, 1437.1, 1381.6, 1188.1, 1117.0, 995.8, 7582.2, 7214.4, 6961.6, NMR (300 MHz, CDCl₃, ppm): 7.3767-7.2227 (m, 10H, CH); 4.7589-4.6965 (dd, 1H, CH, J=3.70Hz, 3.68Hz); 3.8201 (s, 2H, CH₂); 2.9656-2.6927 (m, 2H, CH₂); 2.6927 (s, 1H, OH exchangeable on D₂O shake); 2.3530 (bs, 1H, NH, exchangeable on D₂O shake). Mass (ESMS⁺): 227(M+1), Elemental analysis: (theoretical) C=79.26; H=7.54; N=6.16; (experimental) C=78.26; H=6.94; N=5.99.

Formation of N-benzyl-2-bromo-2-phenylethanamine: Yield: 270 mg (44%), Melting point: 127 °C (IR cm⁻¹): 3045.8, 2962.2, 2863.6, 1603.0, 1506.9, 1405.3, 1297.9, 1226.5, 1093.6, 1055.5, 1015.1, 820.3, 781.9, NMR (300 MHz, CDCl₃, ppm): 7.3769-7.2224 (m, 10H, CH); 4.709-4.6952 (dd, 1H, CH, J=3.70Hz, 3.68Hz); 3.8200 (s, 2H, CH₂); 2.9626-2.6920 (m, 2H, CH₂); 2.3370 (bs, 1H, NH, exchangeable on D₂O shake). Mass (ESMS⁺): 291(M+1), 293(M+3), Elemental analysis: (theoretical) C=62.08; H=5.56; Br=27.53; N=4.83; (experimental) C=61.08; H=4.93; Br=27.49; N=4.80.

Formation of 3-(benzylamino)-2-phenylpropanenitrile: Yield: 350 mg (67%), Melting point: 68-74 °C, IR (cm⁻¹): 3064.9, 2908.7, 2833.2, 2364.6, 1599.3, 1440.5, 1065.3, 1034.1, 920.1, 872.0, 754.2, 697.7, NMR (300 MHz, CDCl₃, ppm): 7.3397-7.1274 (m, 10H, CH); 7.407-4.6962 (dd, 1H, CH, J=3.70Hz, 3.68Hz); 3.8204 (s, 2H, CH₂); 2.9625-2.6924 (m, 1H, CH₂); 2.3376 (bs, 1H, NH, exchangeable on D₂O shake). Mass (ESMS⁺): 237(M+1), Elemental analysis: (theoretical) C=81.32; H=6.62; N=11.85; (experimental) C=80.67; H=6.94; N=11.99.

Boc protection of 2-(benzylamino)-1-phenylethanol: Yield: 11.5 g (78%), Melting point: 115 °C, Mass (ESMS⁺): 327(M+1).
Derivatization of 2-(benzylamino)-1-phenylethanol:

O-acetylation of 2-(benzylamino)-1-phenylethanol:
Yield: 360 mg (63%). Melting point: the compound was viscous so melting point cannot be determined, IR (cm⁻¹): 3020.9, 2361.8, 2339.3, 1731.0, 1601.2, 1481.9, 1375.2, 1216.4, 1045.2, 769.4, NMR (300 MHz, CDCl₃ ppm): 7.3397-7.1274 (m, 10H, CH); 4.7403-4.6962 (dd, 1H, CH₃J=5.2Hz, 4.3Hz); 3.8955 (s, 2H, CH₂); 3.7536 (s, 3H, CH₃); 2.9625-2.6924 (ddd, 2H, CH₂J=4.14Hz, 9.8Hz, 1.6Hz, Mass (ESMS⁺): 270(M+1), Elemental analysis: (theoretical) C=56.58; H=5.72; N=5.99.

O-benzoylation of 2-(benzylamino)-1-phenylethanol:
Yield: 545 mg (87%). Melting point: the compound was viscous so melting point cannot be determined, IR (cm⁻¹): 3451.4, 3021.4, 2411.6, 2363.7, 1721.2, 1565.2, 1525.4, 1390.9, 1215.8, 925.4, 761.2, NMR (300 MHz, CDCl₃ ppm): 8.1662(J=2.8Hz); 7.3849-7.2485(m, 11H, CH); 5.4595 (t, 1H, CH, J=4.53Hz); 2.5480 2.3723(m, 2H, CH₂); 2.1642 (s, 2H, CH₂), Mass (ESMS⁺): 331.2 (M+1), Elemental analysis: (theoretical) C= 78% N=3.91, (experimental) C=78.86; H= 6.94; N= 3.99.

Synthesis of methyl ester of phenylalanine hydrochloride: Yield: 11.4 g (98%), Melting point: 104 °C, IR (cm⁻¹): 3415.6, 3020.8, 2358.9, 1747.2, 1618.6, 1522.1, 1439.5, 1217.0, 1044.7, 929.0, 761.7, NMR (300 MHz, CDCl₃ ppm): 8.0494 (bs, 2H, NH₂); 7.3924-7.1941(m, 5H, CH); 4.2816(t, 1H, CH, J=2.85Hz); 3.6957(s, 3H, CH₃); 3.4994(t, 1H, CH₂), J=0.66Hz); 3.1556(t, 1H, CH, J=11.0Hz) Mass (ESMS⁺): 216.0(M+1), Elemental analysis: (theoretical) C=55.69; H=6.54; N=16.44; (experimental) C=55.60; H=6.69; Cl=16.34; N=6.47.

Synthesis of 2-(3-benzyl-4-carboxyphenylbutanol) benzoic acid: Yield: 6.4 g (78%), Melting point: 156 °C, IR (cm⁻¹): 3319.5, 2954.0, 1718.6, 1625.1, 1545.6, 1227.9, 699.4, NMR (300 MHz, DMSO-d₆ ppm): 7.9729 (t, 1H, CH); 7.5414-7.2067 (m, 8H, CH); 5.0245 (t, 1H, CH, J=11Hz); 4.5953 (bs, 1H, CH₃); 3.7244 (s, 3H, CH₃); 3.2329-3.2040 (dd, 2H, CH₂J=2.37Hz, 2.49Hz), C13NMR (300 MHz, CDCl₃ ppm): 176.2, 174.1, 173.8, 140.3, 139.1, 133.3, 132.6, 132.3, 131.2, 130.3, 129.7, 57.7, 40.18 (CH₂), 32.5 (CH₃) Mass (ESMS⁺): 327.3(M+1), Elemental analysis: (theoretical) C=65.93; H= 5.56; N=4.51 (experimental) C=69.9; H= 5.66; N= 4.61.

Preparation of tryptophan methyl ester hydrochloride: Yield: 11.4 g (93.6%), Melting point = 110 °C, IR (KBr): 3184, 2836, 1738, 1591, 1437, 1347, 1172, 732 cm⁻¹, ESI-MS: m/z 218 [M+1] 1H NMR (CDCl₃, 200MHz): 7.01-7.40 (m, 2H, CH); 6.74-7.09 (m, 2H, CH₂); 3.82(s, 3H, OCH₃); 3.58(s, 2H, CH₂); 1H, Mass (ESMS⁺): 313.3(M+1), Elemental analysis: (theoretical) C=69.22; H=5.16; N=3.83 (experimental) C=69.33; H=5.06; N= 3.69.

Synthesis of 2-(3-(1H-indol-3-yl)-1-methoxy-1-oxopropan-2-ylcarbamoyl) benzoic acid: Yield: 6.4 g (70%), Melting point: 156 °C, IR (cm⁻¹): 3459.6, 3399.1, 3150.1, 3941.1, 2941.1, 2073.8, 2825.5, 2361.8, 1701.8, 1604.2, 1507.7, 1454.1, 1333.5, 1225.7, 1156.7, 1099.4, 1063.6, 814.1, 740.5, 698.8,
Synthesis of 2-(1-carboxy-2-(1H-indol-3-yl) ethylcarbamoyl) benzoic acid: Yield: 6.4 g (78%), Melting point: 168 °C, IR (cm⁻¹): 3446.5, 3323.9, 3148.9, 3060.3, 2995.7, 2829.1, 2753, 1740.1, 1580, 1438.3, 1351.8, 1234.3, 1192.9, 1122.5, 1005.5, 742.5, NMR (300 MHz, CDCl₃, ppm): 9.025(bs, 1H, NHindole); 8.9341(s, 1H, NH); 8.2742(d, 1H, CH, J=7.56Hz); 7.636(t, 1H, CH, J=7.48Hz); 7.5088(d, 1H, CH, J=7.38Hz); 7.5057(d, 1H, CH, J=8.07Hz); 7.4185-7.198(m, 5H, CH); 5.4198(t, 1H, CH, J=2.61Hz); 3.646(s, 3H, CH₃); 3.3295-3.0895(tt, 2H, CH₂, J=1.44Hz, 12.4Hz, Mass (ESMS⁺): 366.1(M+1), Elemental analysis: (theoretical) C=64.33; H= 4.46; N=7.69; (experimental) C=64.93; H=4.95; N=7.65; (experimental) C=64.77; H=4.58; N=7.95.

Initially generated hypothesis suggested Hydrogen bond acceptor (HBA), Hydrophobic (HY), Hydrogen bond donor (HBD) and ring aromatic (RA) to be able to map important features of all of the compounds in the dataset. These features were used to generate 10 predictive hypotheses using the training set molecules. The null, fixed and configuration costs were found to be 147.71, 105.374 and 16.7955 respectively. The total cost range from 146.254 to 180.362 for the ten hypotheses while the difference of null cost and total cost was found to be >20 for the first seven hypotheses out of the 10 generated, indicating that these hypotheses have at least 50-75% probability of representing true correlation in the data (Table 1). The hypotheses 1, 2, 3, 4, &10 contain one HBA, 3 hydrophobic and one ring aromatic while the hypothesis 5, 6, and 7 contains HBA HBD & 2-hydrophobic. The hypotheses 8 contain HBA HBD & 3 hydrophobic. The hypotheses 9 contain HBA & 2 hydrophobic ring aromatic. The cost values, correlation coefficients and different pharmacophoric features for generated hypothesis are reported in Table 2.

Table 1. Results of pharmacophore hypothesis for EGFR inhibitors.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Total cost</th>
<th>Cost difference (Null-Totals)</th>
<th>RMS Deviation</th>
<th>Error Correlation</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118.05</td>
<td>29.66</td>
<td>0.9851</td>
<td>0.1007</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic, HBA, hydrophobic hydrophobic ring aromatic</td>
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<td>2</td>
<td>121.48</td>
<td>26.21</td>
<td>1.0150</td>
<td>0.1033</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
<tr>
<td>3</td>
<td>122.4</td>
<td>25.30</td>
<td>1.1437</td>
<td>0.1045</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
<tr>
<td>4</td>
<td>123.47</td>
<td>24.23</td>
<td>1.1158</td>
<td>0.1012</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
<tr>
<td>5</td>
<td>125.96</td>
<td>21.72</td>
<td>1.2530</td>
<td>0.1066</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
<tr>
<td>6</td>
<td>125.98</td>
<td>21.98</td>
<td>1.2589</td>
<td>0.1059</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
<tr>
<td>7</td>
<td>126.96</td>
<td>20.75</td>
<td>1.2887</td>
<td>0.1045</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
<tr>
<td>8</td>
<td>127.04</td>
<td>20.66</td>
<td>1.2424</td>
<td>0.1072</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
<tr>
<td>9</td>
<td>128.15</td>
<td>19.58</td>
<td>1.2985</td>
<td>0.1037</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
<tr>
<td>10</td>
<td>128.25</td>
<td>19.50</td>
<td>1.2858</td>
<td>0.1049</td>
<td>HBA, hydrophobic, hydrophobic ring aromatic</td>
</tr>
</tbody>
</table>

Mapping Studies: The generated model further subjected to another validation which included prediction of compounds which are not present in the initially designed training and test set. This is done to test whether it can identify other molecules which are active inhibitor of EGFR, this may indicate the true utility of the generated pharmacophore model. In this endeavor the pharmacophore model was tested against drugs which are under clinical trial. The pharmacophore model correctly predicted the activities of these molecules also and established the universal applicability of the generated model. This indicates the high 3D similarity along with important interfeature distances among these molecules. Nevertheless, the mapping clearly shows that the bridged side chain needs further modification to map correctly onto the ring aromatic feature, and this information may help in designing compounds with improved activity. The fit values of these compounds to Hypo-1 along with experimentally derived IC₅₀ values are given in Table 4. The pharmacophore so developed in this case was found to be mapping all ten compounds which are well known HIV-RTase inhibitors. The results of mapping were found to be interesting as the correlation of 0.849 between...
estimated activity and the fit values of Hypothesis-1 and also the results of the correlation of 0.586 between reported activities and estimated by the mapping study further indicates utility of the pharmacophore model. In the case of the mapping of the La compound (83) from the series it was found out that the molecule lacks some of the features from the model derived so mapped and predicted correctly at least active.

Table 2. Structure activity relationship predicted against EGF receptor.

<table>
<thead>
<tr>
<th>Mol</th>
<th>Structure</th>
<th>Activity predicted against EGF receptor (nm)</th>
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References

with raloxifene: results from a 3-year randomized clinical trial. JAMA 282:637–45.