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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

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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.

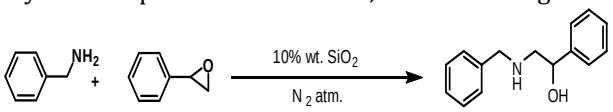
Therapies for osteoporosis are licensed for either prevention or treatment, or both this distinction is somewhat artificial and whether a treatment is used in either way will tend to depend more on the balance between risks and benefits and whether the treatment is acceptable to the subject and cost 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 non vertebral 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 antiosteoporotic agents and QSAR studies on EGFR receptor inhibitors.

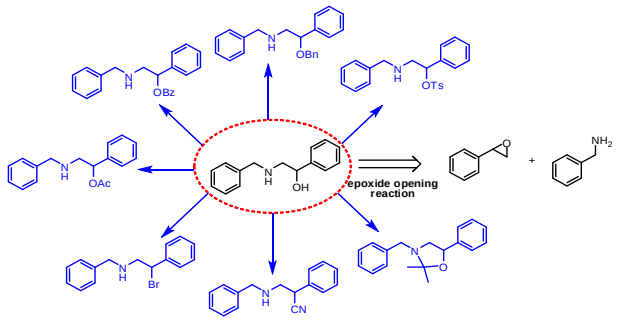
Experimental

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 Dragondroff's spray reagent, ninhydrin reagent, potassium permaganate 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 U.S.A). 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 Quattro II spectrometer. Elemental analyses were carried out on Carlo ERBA CHNS-OEA1108- Elemental Analyzer.

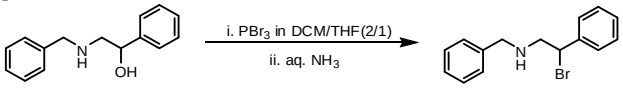
Synthesis of 2-(benzylamino)-1-phenylethanol: In a dry round bottom flask 11 g of benzyl amine was taken along with 12.36 g of styrene oxide under nitrogen atmosphere and added SiO_2 by 10% w of the initial, allowed the reaction mixture to stir over night under nitrogen atmosphere. After completion of the reaction (as per the TLC in the solvent system 9:1 DCM: hexane, R_f =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



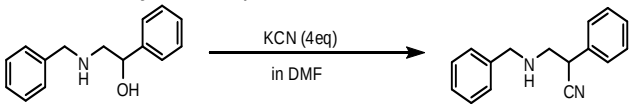


Scheme 1: Functional group transformation for alcoholic group.

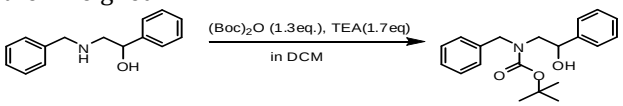
Formation of N-benzyl-2-bromo-2-phenylethanamine: 500mg of 2-(benzyl amino)-1-phenylethanol was dissolved in dry DCM/dry THF (10ml+5ml) then added 0.8ml of phosphorus tribromide dissolved in 10ml of dry DCM dropwise at -20°C with the help of dropping funnel salt formed with the amine part get separated 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/ EtOAc: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_2SO_4 concentrated to obtain the desired product.



Formation of 3-(benzylamino)-2-phenylpropanenitrile: Five hundred mg of 2-(benzylamino)-1-phenylethanol was dissolved in 4ml of dry DMF added 250 mg potassium cyanide and allowed the reaction to stir overnight. After completion of the reaction as indicated by the TLC (1:4/EtOAc:Hex), 20 ml of ice cooled water was added to the reaction mixture the solid separated was filtered and remaining product was obtained by extracting with ethylacetate drying the organic solvent over Na_2SO_4 and concentrated under *vacuo* till the traces of DMF was removed. The crude product was purified by eluting the column with 5% ethylacetate/hexane solution.

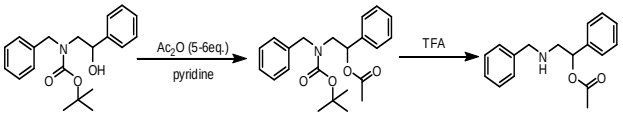


Boc protection of 2-(benzylamino)-1-phenylethanol: Eight g compound 1.1 was taken in a 250 ml flask and was dissolved in 100 ml of dry DCM and then cooled to 0 ˚C then added 9.2 ml of $(Boc)_2O$ (di-tert-butyl carbonate) followed by addition of 7 ml of TEA with the aid of dropping funnel. The reaction was allowed to stir overnight. After the disappearance of initial in TLC taken in pure hexane the reaction mixture was concentrated till the smell of TEA get diminished, added 60ml of water to remove the traces of TEA and extracted the compound with DCM dried over Na_2SO_4 concentrated *in vacuo*, the obtained solid product was dried in a desiccators for overnight and then weighed.



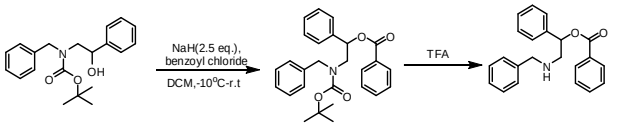
Derivatization of 2-(benzylamino)-1 phenylethanol

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_2SO_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*.

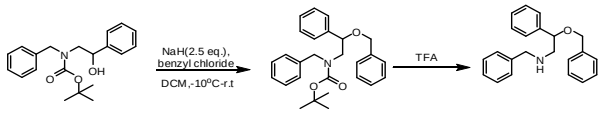


O-benzoylation of 2-(benzylamino)-1-phenylethanol: One hundred and sixty four mg of NaH (2.5 eq. washed with hexane to remove the mineral oil) was taken in 50 ml flask added 500 mg of the Boc protected compound dissolved in 15 ml of dry DCM was added at -10 ˚C and slowly added benzoyl

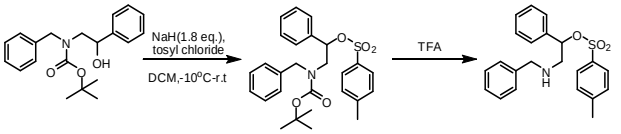
chloride 0.5ml (1.5-2eq.) with vigorous stirring and allowed the reaction mixture to achieve room temperature. After 2.5 h the reaction mixture was concentrated in high vacuum added 60 ml of chilled water and extracted with ethylacetate (3×100 ml) dried the organic layer over Na_2SO_4 concentrated *in 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.*



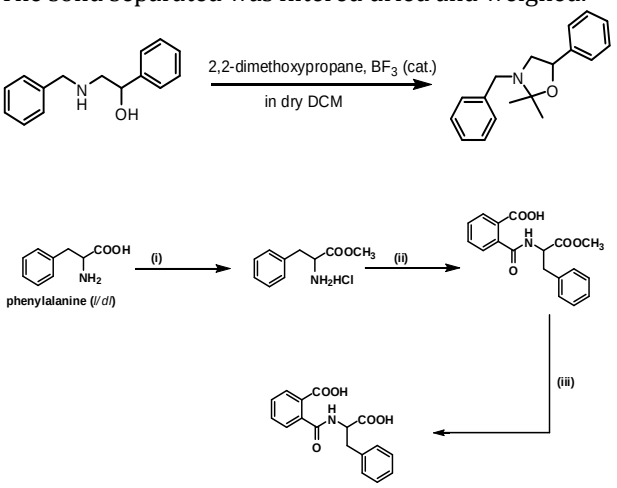
O-benzylation of 2-(benzylamino)-1-phenylethanol: One hundred and sixty four mg of NaH (2.5 eq. washed with hexane to remove the mineral oil) was taken in 50 ml flask added 500 mg of the Boc protected compound dissolved in 15 ml of dry DCM was added at -10 ˚C and slowly added benzyl chloride 0.5 ml (3 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 Na_2SO_4 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.*



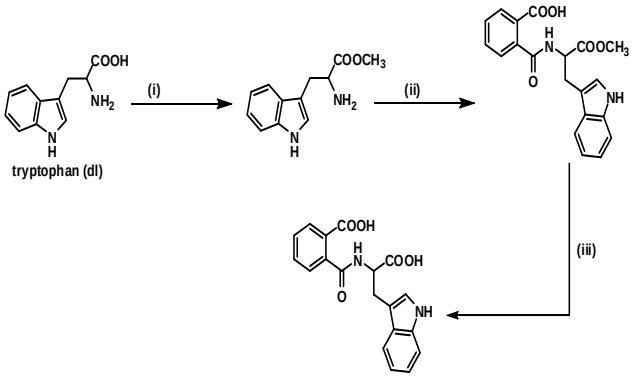
O-tosylation of 2-(benzylamino)-1-phenylethanol: One hundred and thirty four mg of NaH (1.8 eq. washed with hexane to remove the mineral oil) was taken in 50 ml flask added 500 mg of the Boc protected compound dissolved in 15 ml of dry DCM was added at -10 ˚C and added tosyl chloride 0.4 g (1.5 eq.) 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 Na_2SO_4 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.

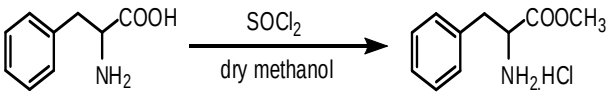


Scheme 2. Reagents and conditions: (i) $SOCl_2$(thionyl chloride)1.3 eq, Dry methanol; (ii) phthalic anhydride(1.2 eq.), TEA (2.2 eq) in DCM; (iii) LiOH (3 eq.), THF+methanol+water (4:1:1)

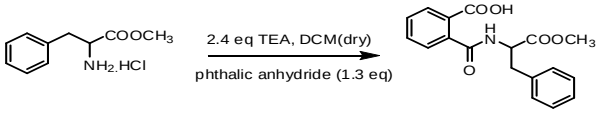


Scheme 3. Reagents and conditions: (i) $SOCl_2$(thionyl chloride)1.3eq, Dry methanol; (ii)phthalic anhydride(1.2 eq.), TEA (2.2 eq) in DCM; (iii) LiOH (3 eq.), THF+methanol+water (4:1:1)

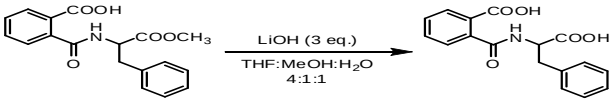
Synthesis of methyl ester of phenylalanine hydrochloride: Ten g of DL phenylalanine was taken in a 500 ml flask and added 250 ml absolute methanol and brought the temperature of the suspension thus obtained to 0-5 °C. Then added (5+1 ml) thionyl chloride to the reaction mixture and allowed to attain the room temperature then stirred for 10-14 h. On completion of the reaction as per the TLC product was filtered under suction.



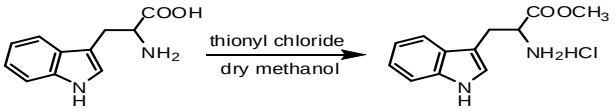
Synthesis of 2-(3-benzyl-4-methoxy-4-oxobutanoyl) benzoic acid: Five g of 2-(3-benzyl-4-methoxy-4-oxobutanoyl) benzoic acid was taken in 250 ml flask charged with dry DCM (100 ml) and cooled at 0 °C and added 8.1 ml TEA (2.4 eq) after 15 min of stirring, 4.1 g of phthalic anhydride (1.3 eq) was added to the reaction mixture. Then, allowed the reaction to stir overnight. After the completion of the reaction 100ml 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 Na_2SO_4 then concentrated *in vacuo.*



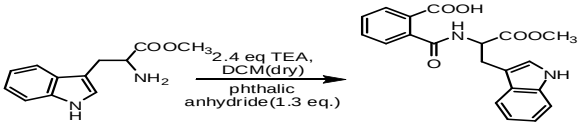
Synthesis of 2-(3-carboxy-4-phenylbutanoyl) benzoic acid: Four g of 2-(3-benzyl-4-methoxy-4-oxobutanoyl) benzoic acid was taken in a 100 ml R.B. flask and added trisolvent (THF:MeOH:H_2O = 8ml:2ml:2ml) system to dissolve the initial compound then added 950 mg of lithium hydroxide (3 eq.) and allowed the reaction to stir at room temperature for 1.5 h. 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 (60ml×3), dried over anhyd. Na_2SO_4 and concentrated *in vacuo.*



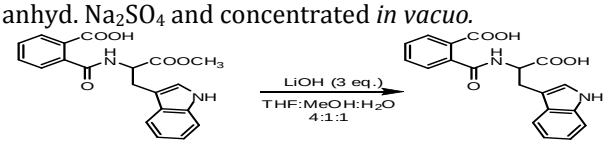
Preparation of tryptophan methyl ester hydrochloride: Ten mg of tryptophan was taken in a 1000 ml flask and added 750 ml absolute methanol and brought the temperature of the suspension thus obtained to 0-5 °C. Then added (5ml+1ml) thionyl chloride to the reaction mixture and allowed to attain the room 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 100ml 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 Na_2SO_4 then concentrated *in vacuo.*



Synthesis of 2-(1-carboxy-2-(1H-indol-3-yl) ethylcarbamoyl) benzoic acid: Four g of 2-(3-benzyl-4-methoxy-4-oxobutanoyl) benzoic acid was taken in a 100 ml flask and added trisolvent (THF:MeOH:H_2O = 8ml:2ml:2ml) system to dissolve the initial compound then added 950 mg of lithium hydroxide (3 eq.) and allowed the reaction to stir at room temperature for 1.5 h. 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 (60ml × 3), dried over anhyd. Na_2SO_4 and 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, 758.2, 721.4, 696.1, NMR (300 MHz, $CDCl_3$, ppm): 7.3767-7.2227 (m, 10H, CH); 4.7589-4.6965 (dd, 1H, CH, J=3.70Hz, 3.68Hz); 3.8201 (s, 2H, CH_2); 2.9656-2.6927 (m, 2H, CH_2); 2.6927 (s, 1H, OH exchangeable on D_2O shake); 2.3530 ((bs, 1H, NH, exchangeable on D_2O shake), Mass ($ESMS^{+1}$): 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, $CDCl_3$, ppm): 7.3769-7.2224 (m, 10H, CH); 4.709-4.6952 (dd, 1H, CH, J=3.70Hz, 3.68Hz); 3.8200 (s, 2H, CH_2); 2.9626-2.6920 (m, 2H, CH_2); 2.3370 (bs, 1H, NH, exchangeable on D_2O shake), Mass ($ESMS^{+1}$): 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, $CDCl_3$, ppm): 7.3397-7.1274 (m, 10H, CH); 4.703-4.6962 (dd, 1H, CH, J=3.70Hz, 3.68Hz); 3.8204 (s, 2H, CH_2); 2.9625-2.6924 (m, 2H, CH_2); 2.3375 (bs, 1H, NH, exchangeable on D_2O shake), C^{13}NMR (300 MHz, $CDCl_3$, ppm): 140.3, 139.1, 134.8, 133.3, 132.6, 130.3,129.7, 139.1, 56.8, 40.1, 32.5 Mass ($ESMS^{+1}$): 237(M+1), Elemental analysis: (theoretical) C=81.32; H=6.82; 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 ($ESMS^{+1}$): 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, $CDCl_3$, ppm):7.3397-7.1274 (m, 10H, CH); 4.7403-4.6962 (dd, 1H, CH,J=5.2Hz, 4.3Hz); 3.8955(s, 2H, CH_2); 3.7536 (s, 3H,CH_3); 2.96.25-2.6924 (ddd, 2H, CH_2, J=4.14Hz, 9.8Hz, 1,6Hz, Mass ($ESMS^{+1}$): 270(M+1, Elemental analysis: (theoretical) C=75.81; H=7.11; N=5.20; (experimental) C=71.26; H= 6.94; 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, $CDCl_3$, ppm):8.1662-7.9054 (dd, 2H, CH, J=3.4Hz); 7,4653 (d, 2H, CH, J=8.28Hz); 7.3489-7.2485(m, 11H, CH); 5.4595 (t, 1H, CH, J=4.53Hz); 2.5480 2.3723 (m, 2H, CH_2); 2.1642 (s, 2H, CH_2)**,** Mass ($ESMS^{+1}$): 331.2 (M+1)**,** Elemental analysis: (theoretical) C= 79.73; H= 6.39; N= 4.23; (experimental) C=78.86; H= 6.94; N= 3.99.

O-benzylation of 2-(benzylamino)-1-phenylethanol: Yield: 349 mg (54%), Melting point: the compound was viscous so melting point cannot be determined, IR (cm^{-1}): 3447.3, 3020.9, 2368.8, 1638.2, 1318.9, 1216.4, 1019.8, 832.1, 762.9, 670.4 NMR (200 MHz, $CDCl_3$, ppm): 7.3769-7.2227 (m, 15H, CH); 5.2800 (s, 2H, CH_2); 4.7100 (dd, 1H, CH, J=5.72Hz, 5.64Hz); 3.8200(s, 2H, CH_2); 2.9626-2.6927(ddd, 2H, CH_2, J=4.3Hz, 6.2Hz); 2.3370(bs, 1H, NH) Mass ($ESMS^{+1}$): 318(M+1) Elemental analysis: (theoretical) C= 83.24; H= 7.30; N= 4.41;; (experimental) C=78.00; H= 6.94; N=3.99.

O-tosylation of 2-(benzylamino)-1-phenylethanol: Yield: 350 mg (62%), Melting point: the compound was viscous so melting point cannot be determined**,** IR (cm^{-1}): 3387.7, 3031.3, 2878.9, 1696.0, 1655.1, 1601.6, 1505.1, 1452.1, 1412.1, 1304.7, 1279.7, 1231.1, 1158.1, 1019.3, 816.5, 792.0, 759.9, 700.1 NMR (300 MHz, $CDCl_3$, ppm): 7.4763(d, 2H, CH, J=8.49Hz); 7.3558-7.2489(m, 12H, CH); 6.0071(t, 1H, CH, J=3.6Hz); 3.82(s, 2H, CH_2); 2.5408-2.3732(m, 2H, CH_2); 2.2553(s, 3H, CH_3); 1.8(bs, 1H, NH) Mass ($ESMS^{+1}$): 382.4(M+1) Elemental analysis: (theoretical) C= 69.26; H= 6.08; N= 3.67; S= 8.41; (experimental) C=68.26; H= 6.44; N= 3.99; S=8.54.

Synthesis of 3-benzyl-2,2-dimethyl-5-phenyloxazolidine: Yield: 400 mg (70%), Melting point: the compound was viscous so melting point cannot be determined, IR (cm^{-1}): 3032.5, 2863.0, 1604.7, 1507.7, 1453.0, 1298.9, 1225.5, 1185.3, 1109.5, 1056.4, 1022.3, 852.3, 816.7, 740.2, 700.7, NMR (300 MHz, $CDCl_3$, ppm): 7.5334-7.4488(m, 4H, CH); 7.3098-7.1910(m, 6H, CH); 4.2779(t, 1H, CH, J=3.24Hz); 3.4479(s, 2H, CH_2); 3.3445-3.2699(m, 1H, CH_2); 3.2255-3.1587(m, 1H, CH_2); 1.2483(s, 6H, CH_3), Mass ($ESMS^{+1}$): 268(M+1), Elemental analysis: (theoretical) C=80.86; H= 7.92; N= 5.24; (experimental) C=81.06; H= 7.94; N= 5.09.

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, $CDCl_3$, ppm): 8.0494 (bs, 2H, NH_2); 7.3924-7.1941(m, 5H, CH); 4.2816(t, 1H, CH, J=2.85Hz); 3.6957(s, 3H, CH_3); 3.4994(t, 1H, CH_2, J=0.66Hz); 3.1556(t, 1H, CH_2, J=11.0Hz) Mass ($ESMS^{+1}$): 216.0(M+1)**,** Elemental analysis: (theoretical) C=55.69; H=6.54; Cl=16.44; N=6.49; (experimental) C=55.60; H=6.49; Cl=16.34; N=6.47.

Synthesis of 2-(3-benzyl-4-methoxy-4-oxobutanoyl) 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-d_6, ppm): 7.9729 (t, 1H, CH); 7.5414-7.2067 (m, 8H, CH); 5.0245 (t, 1H, CH, J=11Hz); 4.4593 (bs, 1H, CH3); 3.7244 (S, 3H, CH_3); 3.2329-3.2040 (dd, 2H, CH_2, J=2.37Hz, 2.49Hz)**,** C13NMR (300 MHz, $CDCl_3$, 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 (CH_2), 32.5 (CH_2) Mass ($ESMS^{+1}$): 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.

Synthesis of 2-(3-carboxy-4-phenylbutanoyl) benzoic acid: Yield: 6.4 gm, 78%, Melting point: 168 ^oC, IR (cm^{-1}): 3447.3, 3020.9, 2363.8, 1638.2, 1215.4, 1019.7, 937.1, 762.9, NMR (300 MHz, $CDCl_3$, ppm): 7.9280 (t, 1H, CH); 7.5334-7.4498 (m, 8H, CH); 7.3098-7.1910 (m, 6H, CH); 4.9779 (t, 1H, CH, J=5.9Hz); 3.3445-3.1587 (m, 2H, CH_2); 1.2483(s, 1H, exchangeable on D_2O shake), C13NMR (300 MHz, $CDCl_3$, ppm): 142.8, 140.3, 128.9, 128.7, 127.9, 127.5, 126.2, 72.2, 56.9, 53.9, Mass ($ESMS^{+1}$): 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.

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+]. 1H NMR (CDCl3, 200MHz): 7.01-7.40 (m, 5H, CH) 4.07 (s, 1H, Ch), 3.77 (t, 3H, CH_3), 3.12 (s, 1H), 3.03(s,1H), 1.79(s, 1H)**,** Elemental analysis: (theoretical) C=56.58; H=5.94; N=11.00. (Experimental): C= 56.86; H=6.05; N=11.19.

Synthesis of 2-(3-(1H-indol-3-yl)-1-methoxy-1-oxopropan-2-ylcarbamoyl) benzoic acid: Yield: 6.4 g (70%)**,** Melting point: 156 ^oC**,** 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**,**

NMR (300 MHz, DMSO-d_6, ppm): 9.025(bs, 1H, NH_{indole}); 8.9314(s, 1H, NH); 8.2736(d, 1H, CH, J=7.56Hz); 7.6306(t, 1H, CH, J=7.38Hz); 7.5057(d, 1H, CH, J=8.07Hz); 7.4185-7.1798(m, 5H, CH); 5.4198(t, 1H, CH, J=2.61Hz); 3.6464(s, 3H, CH_3); 3.3295-3.0895(tt, 2H, CH_2, J=1.44Hz, 12.4Hz, Mass ($ESMS^{+1}$): 366.1(M+1), Elemental analysis: (theoretical) C= 65.57; H= 4.95; N=7.65; (experimental) C=64.93; H= 4.66; O= 7.61.

Synthesis of 2-(1-carboxy-2-(1H-indol-3-yl) ethylcarbamoyl) benzoic acid: Yield: 6.4 g (78%), Melting point: 168 ^oC, IR (cm^{-1}): 3446.5, 3323.9, 3148.9, 3060.3, 2995.7, 3829.1, 2753, 1740.1, 1580, 1438.3, 1351.8, 1234.3, 1192.9, 1122.5, 1005.5, 742.5, NMR (300 MHz, $CDCl_3$, ppm): 9.0252(bs, 1H, NH_{indole}); 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=8.06Hz); 7.4158-7.1789(m, 5H, CH); 5.4188(t, 1H, CH, J=2.61Hz); 3.3274-3.0858(tt, 2H, CH_2, J=1.44Hz, 12.4Hz), Mass ($ESMS^{+1}$): 352.3 (M+1), Elemental analysis: (theoretical) C= 64.77; H=4.58; N=7.95 (experimental) C=64.33; H= 4.46; N= 7.69.

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.

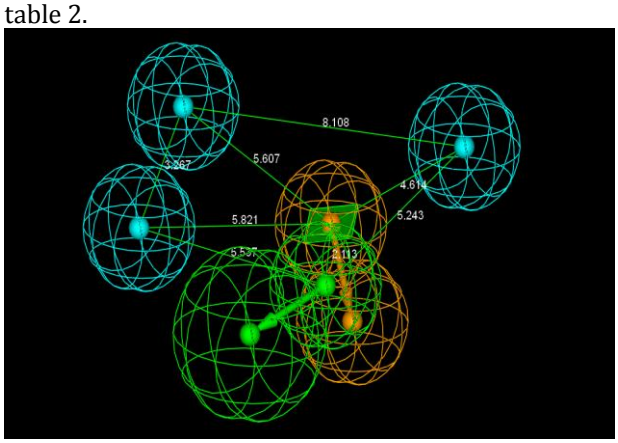


Figure 1. QSAR Studies.

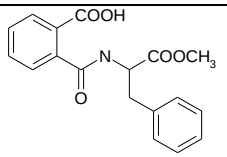
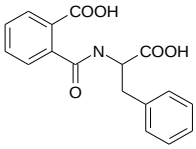
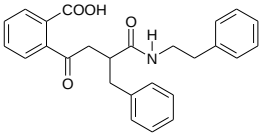
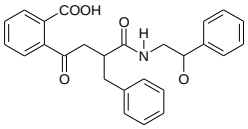
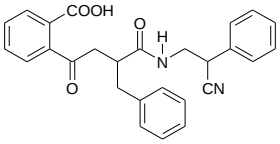
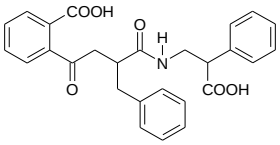
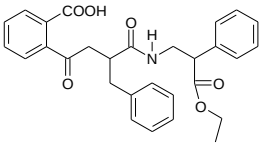
Table 1. Results of pharmacophore hypothesis for EGFR inhibitors.

Hypothesis	Total cost	Cost difference (Null[a] – Total)	RMS Deviation	Error	Correlation	Features[b]
1	118.05	29.66	0.9851	100.071	0.8892	HBA, hydrophobic, hydrophobic, hydrophobic ring aromatic,
2	121.48	26.21	1.1050	103.33	0.8584	HBA,Hydrophobic hydrophobic hydrophobic ring aromatic
3	122.4	25.30	1.1437	104.459	0.8472	HBA, hydrophobic, hydrophobic ,hydrophobic ring aromatic
4	123.47	24.23	1.1658	105.123	0.8410	HBA, hydrophobic hydrophobic hydrophobic ring aromatic
5	125.96	21.72	1.2530	107.866	0.8134	HBA, hydrophobic hydrophobic, hbd
6	125.98	21.98	1.2589	108.059	0.8112	HBA ,hydrophobic hydrophobic, hbd
7	126.96	20.75	1.2887	109.045	0.8010	HBA, hydrophobic hydrophobic, hbd
8	127.04	20.66	1.2424	107.521	0.8177	HBA, hydrophobic hydrophobic ring aromatic
9	128.15	19.58	1.2985	109.375	0.7997	HBA, hydrophobic hydrophobic hydrophobic ring aromatic
10	128.24	19.50	1.2858	108.949	0.8030	HBA hydrophobic hydrophobic hydrophobic ring aromatic

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 IC_{50} 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 as least active.

Table 2. Structure activity relashionship predicted against EGF receptor.

Mol	Structure	Activity predicted against EGF receptor (nm)
F1		396.964
F2		789.682
F3		265.882
F4		17.958
F5		427.891
F6		431.905
F7		175.247

References

1. Burge, R.T., Worley, D., Johansen, A. et al. (2001). The cost of osteoporotic fractures in the UK: projections for 2000–2020. J Drug Assessment 4:71–160.
2. Kanis, J., Brazier, J., Stevenson, M. et al. (2002). Treatment of established osteoporosis: a systematic review and cost-utility analysis. Health Tech Assessment 6(29).
3. Lawrence, T.M., White, C.T., Wenn, R. et al. (2005). The current hospital costs of treating hip fractures. Injury 36:88–91.
4. Department of Health (2005). Hospital Episode Statistics (England). http://www.hesonline (accessed 27 March 2006).
5. Elliot-Gibson, V., Bogoch, E.R., Jamal, S.A. et al. (2004). Practice patterns in the diagnosis and treatment of osteoporosis after a fragility fracture: a systematic review. Osteoporos Int 15:767–78.
6. Brankin, E., Caroline, M. and Munro R (2005). Closing the osteoporosis management gap in primary care: a secondary prevention of fracture programme Curr Med Res Opin 21:425–82.
7. Current Therapies for Osteoporosis 205 7. No authors listed (1993). Consensus development conference: diagnosis, prophylaxis,
and treatment of osteoporosis. Am J Med 94:646–50.
8. Faulkner, K.G. (2005). The tale of the T-score: review and perspective. Osteoporos Int
16:347–52.
9. Masud, T. and Francis, R.M. (2000). The increasing use of peripheral bone densitometry. BMJ 321:396–8.
10. Stone, K., Seeley, D., Lui, L. et al. (2003). BMD at multiple sites and risk of fracture of multiple types: long-term results from the study of osteoporotic fractures. J Bone Miner Res 18:1947–54.
11. Schuit, S.C.E., van der Klift, M., Weel, A.E.A.M. et al. (2004). Fracture incidence and
association with bone mineral density in elderly men and women: the Rotterdam Study. Bone 34:195–202.
12. Heaney, R.P. (2003). Is the paradigm shifting? Bone 33:457–65.
13. Cauley, J.A., Seeley, D.G., Ensrud, K. et al. (1995). Estrogen replacement therapy and fractures in older women. Ann Intern Med 122:9–16.
14. Gallagher, J.C., Rapuri, P.B., Haynatzki, G. et al. (2002). Effect of discontinuation of estrogen, calcitriol, and the combination of both on bone density and bone markers. J Clin Endocrinol Metab 87:4914–23.
15. Writing Group for the Women's Health Initiative (2002). I. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 288:321–33.
16. Anderson, G.L., Limacher, M., Assaf, A.R. et al. (2004). Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 291:1701–12.
17. Stefanick, M.L., Anderson, G.L., Margolis, K.L. et al. (2006). Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. JAMA 295:1647–57.
18. Ettinger, B., Black, D.M., Mitlak, B.H. et al. (1999). Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated

with raloxifene: results from a 3-year randomized clinical trial. JAMA 282:637–45.
19. Cummings, S.R., Eckert, S., Krueger, K.A. et al. (1999). The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. JAMA 281:2189–97.
20. Riggs, B.L. and Hartmann, L.C. (2003). Selective estrogen-receptor modulators— mechanisms of action and application to clinical practice. N Engl J Med 348:618–29.
21. Cauley, J.A., Norton, L., Lippman, M.E. et al. (2001). Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-year results from the MORE trial. Breast Cancer Res Treat 65:125–34.
22. Barrett-Connor, E., Grady, D., Sashegyi, A. et al. (2002). Raloxifene and cardiovascular events in osteoporotic postmenopausal women: four-year results from the MORE (Multiple Outcomes of Raloxifene Evaluation) randomized trial. JAMA 287:847–57.
23. van Staa, T.P., Abenhaim, L. and Cooper, C. (1998). Use of cyclical etidronate and prevention of non-vertebral fractures. Rheumatology 37:87–94.
24. Department of Health (2005). Prescription Cost Analysis.
http://www.ic.nhs.uk/pubs/prescostanalysis2005 [accessed 20 April 2006].
25. Black, D.M., Cummings, S.R., Karpf, D.B. et al. (1996). Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Lancet 348:1535–41.
26. Cummings, S.R., Black, D.M., Thompson, D.E. et al. (1998). Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the Fracture Intervention Trial. JAMA 280:2077–82.
27. Orwoll, E., Ettinger, M., Weiss, S. et al. (2000). Alendronate for the treatment of osteoporosis in men. N Engl J Med 343:604–10.
28. Saag, K.G., Emkey, R., Schnitzer, T.J. et al. (1998). Alendronate for the prevention and treatment of glucocorticoid-induced osteoporosis. N Engl J Med 339:292–9.
29. Rizzoli, R., Greenspan, S.L., Bone, G., 3rd et al.; The Alendronate Once Weekly Study Group (2002). Two-year results of once-weekly administration of alendronate 70 mg for the treatment of postmenopausal osteoporosis. J Bone Miner Res 17:1988–96.
30. Cranney, A., Wells, G., Willan, A. et al. (2002). II. Meta-analysis of alendronate for the treatment of postmenopausal women. Endocr Rev 23:508–16.
31. Bone, H.G., Hosking, D., Devogelaer, J.-P. et al. (2004). Ten years' experience with alendronate for osteoporosis in postmenopausal women. N Engl J Med 350:1189–99.
32. Harris, S.T., Watts, N.B., Genant, H.K. et al. (1999). Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. JAMA 282:1344–52.
33. Reginster, J.Y., Minne, H.W., Sorensen, O.H. et al. (2000). Randomized trial of the effects of risedronate on vertebral fractures in women with established postmenopausal osteoporosis. Osteoporos Int 11:83–91.
34. Sorensen, O.H., Crawford, G.M., Mulder, H. et al. (2003). Long-term efficacy of risedronate: a 5-year placebo-controlled clinical experience. Bone 32:120–6.
35. Ste-Marie, L.G., Sod, E., Johnson, T. et al. (2004). Five years of treatment with risedronate and its effects on bone safety in women with postmenopausal osteoporosis. Calcif Tiss Int 75(6):469–76.
36. Mellstrom,D.D., Sorensen,O.H., Goemaere, S. et al. (2004). Seven years of treatment with risedronate in women with postmenopausal osteoporosis. CalcifTiss Int 75(6):462–8.
37. Cranney, A., Tugwell, P., Adachi, J. et al. (2002). III. Meta-analysis of risedronate for the treatment of postmenopausal osteoporosis. Endocr Rev 23:517–23.
38. Boonen, S., McClung, M.R., Eastell, R. et al. (2004). Safety and efficacy of risedronate in reducing fracture risk in osteoporotic women aged 80 and older: implications for the use of antiresorptive agents in the old and oldest old. JAm Geriatr Soc 52:1832–9.
39. Cohen, S., Levy, R.M., Keller, M. et al. (1999). Risedronate therapy prevents corticosteroid-induced bone loss: a twelve-month, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. Arthritis Rheum 42:2309–18.
40. Reid, D.M., Hughes, R.A., Laan, R.F.J.M. et al. (2000). Efficacy and safety of daily risedronate in the treatment of corticosteroid-induced osteoporosis in men and women: a randomized trial. J Bone Miner Res 15:1006–13.
41. Wallach, S., Cohen, S., Reid, D.M. et al. (2000). Effects of risedronate treatment on bone density and vertebral fracture in patients on corticosteroid therapy. Calcif Tiss Int 67:277–85.
42. Harris, S.T., Watts, N.B., Li, Z. et al. (2004). Two-year efficacy and tolerability of risedronate once a week for the treatment of women with postmenopausal osteoporosis. Curr Med Res Opin 20:757–64.
43. Reid, D.M., Hosking, D., Kendler, D. et al. (2006). Alendronic acid produces greater effects than risedronic acid on bone density and turnover in postmenopausal women with osteoporosis: results of FACTS1-International. Clin Drug Invest 26:63–74.
44. Taggart, H., Bolognese, M.A., Lindsay, R.L. et al. (2002). Upper gastrointestinal safety of risedronate: a pooled analysis. Mayo Clinic Proc 77:262–70.
45. Chesnut, C., 3rd, Skag, A., Christiansen, C. et al. (2004). Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. J Bone Miner Res 19:1241–9.

46. Adami, S., Felsenberg, D., Christiansen, C. et al. (2004). Efficacy and safety of ibandronate given by intravenous injection once every 3 months. Bone 34:881–9.
47. Epstein, S., Delmas, P.D., Emkey, R. et al. (2006). Oral ibandronate in the management of postmenopausal osteoporosis: Reviewof upper gastrointestinal safety. Maturitas 54:1–10.
48. Neer, R.M., Arnaud, C.D., Zanchetta, J.R. et al. (2001). Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 344:1434–41.
49. Rubin, M.R., Cosman, F., Cosman, F. et al. (2002). The anabolic effects of parathyroid hormone. Osteoporos Int 13:267–77.
50. Jiang, Y., Zhao, J.J., Mitlak, B.H. et al. (2003). Recombinant human parathyroid hormone (1–34) [Teriparatide] improves both cortical and cancellous bone structure. J Bone Miner Res 18:1932–41.
51. Ettinger, B., San Martin, J., Crans, G. et al. (2004). Differential effects of Teriparatide on BMD after treatment with raloxifene or alendronate. J Bone Miner Res 19:745–51.
52. Lane, N.E., Sanchez, S., Modin, G.W. et al. (1998). Parathyroid hormone treatment can reverse corticosteroid-induced osteoporosis. Results of a randomized controlled clinical trial. J Clin Invest 102:1627–33.
53. Orwoll, E.S., Scheele, W.H., Paul, S. et al. (2003). The effect of teriparatide [Human Parathyroid Hormone (1–34)] therapy on bone density in men with osteoporosis. J Bone Miner Res 18:9–17.
54. Marie, P.J., Ammann, P., Boivin, G. et al. (2001). Mechanisms of action and therapeutic potential of strontium in bone. Calcif Tiss Int 69:121–9.
55. Marie, P.J. (2005). Strontium ranelate: a novel mode of action optimizing bone formation and resorption. Osteoporos Int 16:S7–S10.
56. Nielsen, S.P., Slosman, D., Sorensen, O.H. et al. (1999). Influence of strontium on bone
mineral density and bone mineral content measurements by dual X-ray absorptiometry. J Clin Densitom 2:371–9.
57. Meunier, P.J., Roux, C., Seeman, E. et al. (2004). The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. N Engl J Med 350:459–68.
58. Reginster, J.Y., Seeman, E., DeVernejoul, M.C. et al. (2005). Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausalwomen with osteoporosis: treatment of peripheral osteoporosis (TROPOS) study. J Clin Endocrinol Metab 90:2816 22.
59. Roux, C., Reginster, J.-Y., Fechtenbaum, J. et al. (2006). Vertebral fracture risk reduction with strontium ranelate in women with postmenopausal osteoporosis is independent of baseline risk factors. J Bone Miner Res 21:536–42.
60. Committee for Proprietary Medicinal Products (CPMAP). European Public Assessment Report. Protelos: scientific discussion [online] http://www.emea.eu.int/
humandocs/PDFs/EPAR/protelos/121604en6.pdf
(accessed 18 April 2006). 61. Heaney, R.P. (2000). Calcium, dairy products and osteoporosis. J Am Coll Nutr 19:83S–99.
62. Dawson-Hughes, B., Harris, S.S., Krall, E.A. et al. (1997). Effect of calcium and vitamin D supplementation on bo ne density in men and women 65 years of age or older. N Engl J Med 337:670–6.
63. Cumming, R.G. and Nevitt, M.C. (1997). Calcium for prevention of osteoporotic fractures in postmenopausal women. J Bone Miner Res 12:1321–9.
64. Department of Health (1998). Nutrition and bone health: with particular reference to calcium and vitaminD. Report on Health and Social Subjects, 49. London: Department of Health.
65. Porthouse, J., Cockayne, S., King, C. et al. (2005). Randomised controlled trial of calcium and supplementation with cholecalciferol (vitamin D3) for prevention of fractures in primary care. BMJ 330:1003–6.
66. The Record Trial Group (2005). Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. Lancet 365:1621–8.
67. Jackson, R.D., LaCroix, A.Z., Gass, M. et al. (2006). Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 354:669–83.
68. Michaelsson, K., Melhus, H., Bellocco, R. et al. (2003). Dietary calcium and vitamin D
intake in relation to osteoporotic fracture risk. Bone 32:694–703.
69. Avenell, A., Gillespie, W., Gillespie, L. et al. (2005). Vitamin D and vitamin D analogues for preventing fractures associated with involutional and post-menopausal osteoporosis. The Cochrane Database of Systematic Reviews Issue 3. CD000227.
70. Prince, R.L., Devine, A., Dhaliwal, S.S. et al. (2006). Effects of calcium supplementation on clinical fracture and bone structure: results of a 5-year, double blind, placebo controlled trial in elderly women. Arch Intern Med 166:869–75.
71. Brennan, J., Johansen, A., Butler, J. et al. (2003). Place of residence and risk of fracture in older people: a population-based study of over 65-year-olds in Cardiff. Osteoporos Int 14:515–19.
72. Cali, C.M. and Kiel, D.P. (1995). An epidemiologic study of fall-related fractures among institutionalized older people. J Am Geriatr Soc 43:1336–40.
73. Chapuy, M.C., Arlot, M.E., Duboeuf, F. et al. (1992). Vitamin D3 and calcium to prevent hip fractures in the elderly women. N Engl J Med 372:1637–42.
74. Chapuy, M.C., Arlot, M.E., Delmans, P.D. et al. (1994). Effect of calcium and cholecalciferol treatment for three years on hip fractures in elderly women. BMJ 308:1081–2.

75. Lilliu, H., Pamphile, R., Chapuy, M.-C. et al. (2003). Calcium-vitamin D3 supplementation is cost-effective in hip fractures prevention. Maturitas 44:299–305.
76. Lips, P., Graafmans, W.C., Ooms, M.E. et al. (1996). Vitamin D supplementation and fracture incidence in elderly persons: a randomized, placebo-controlled clinical trial. Ann Intern Med 124:400–6.
77. Flicker, L., MacInnis, R.J., Stein, M.S. et al. (2005). Should older people in residential care receive vitamin D to prevent falls? Results of a randomized trial. J Am Geriatr Soc 53:1881–8.
78. Bischoff-Ferrari, H.A., Willett, W.C., Wong, J.B. et al. (2005). Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA 293:2257–64.
79. Dawson-Hughes, B., Heaney, R.P., Holick, M.F. et al. (2005). Estimates of optimal vitamin D status. Osteoporos Int 16:713–16.
80. MacLaughlin, J. and Holick, M.F. (1985). Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest 76:1536–8.
81. Holick, M.F., Siris, E.S., Binkley, N. et al. (2005). Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 90:3215–24.
82. Simonelli, C., Weiss, T.W., Morancey, J. et al. (2005). Prevalence of vitamin D inadequacy in a minimal trauma fracture population. Curr Med Res Opin 21:1069–74.
83. Seton, M., Jackson, V., Lasser, K.E. et al. (2005). Low 25-hydroxyvitamin D and osteopenia are prevalent in persons _ 55 yr with fracture at any site: a prospective, Current Therapies for Osteoporosis 209
observational study of persons fracturing in the community. J Clin Densitom
8:454–60.
84. Dixon, T., Mitchell, P., Beringer, T. et al. (2006). An overview of the prevalence of 25-hydroxy-vitamin D inadequacy amongst elderly patients with or without fragility fracture in the United Kingdom. Curr Med Res Opin 22:405–15.
85. Bischoff-Ferrari, H.A., Orav, E.J. and Dawson-Hughes, B. (2006). Effect of cholecalciferol plus calcium on falling in ambulatory older men and women: a 3-year randomized controlled trial. Arch Intern Med 166:424–30.
86. Youm, T., Koval, K.J. and Kummer, F.J., Zuckerman, J.D. (1999). Do all hip fractures result from a fall? Am J Orthop 28:190–4.
87. Royal College of Physicians and the Bone and Tooth Society of Great Britain (2000). Osteoporosis. Clinical guidelines for prevention and treatment. Update on pharmacological interventions and an algorithm for management. London: Royal College of Physicians.
88. Scottish Intercollegiate Guidelines Network (2003). Management of osteoporosis: National clinical guideline 71. http://www.sign.ac.uk/guidelines/fulltext/71/index.html (accessed 7 April 2006).
89. Department of Health (2001). National Service Framework for Older People. London: Department of Health.
90. Tilyard, M.W., Spears, G.F., Thomson, J. et al. (1992). Treatment of postmenopausal osteoporosis with calcitriol or calcium. N Engl J Med 326:357–62.
91. National Institute for Health and Clinical Excellence (2005). Bisphosphonates (alendronate, etidronate, risedronate), selective oestrogen receptor modulators (raloxifene) and parathyroid hormone (teriparatide) for the secondary prevention of osteoporotic fragility fractures in postmenopausal women. Technology appraisal guidance 87. London: NICE. Available at http://www.nice.org.uk/page=aspx?o=TA087guidance accessed 23/11/06
92. Tinetti, M.E., Speechley, M. and Ginter, S.F. (1988). Risk factors for falls among elderly persons living in the community. N Engl J Med 319:1701–7.
93. Pluijm, S., Smit, J., Tromp, E. et al. (2006). A risk profile for identifying communitydwelling elderly with a high risk of recurrent falling: results of a 3-year prospective study. Osteoporos Int 17:417–25.
94. Geusens, P., Autier, P., Boonen, S. et al. (2002). The relationship among history of falls, osteoporosis, and fractures in postmenopausal women. Arch Phys Med Rehabil 83:903–6.
95. Chang, J.T., Morton, S.C., Rubenstein, L.Z. et al. (2004). Interventions for the prevention of falls in older adults: systematic review and meta-analysis of randomised clinical trials. BMJ 328:680.
96. Gillespie, L.D., Gillespie, W.J., Robertson, M.C. et al. (2003). Interventions for preventing falls in elderly people. The Cochrane Database of Systematic Reviews Issue 4. CD000340.
97. Feldstein, A., Elmer, P., Nichols, G. et al. (2005). Practice patterns in patients at risk for glucocorticoid-induced osteoporosis. Osteoporos Int 16:2168–74.
98. Aspray, T.J., Stevenson, P., Abdy, S.E. et al. (2006). Low bone mineral density measurements in care home residents—a treatable cause of fractures. Age Ageing 35:37–41.