Molecular modeling studies and synthesis of fused heterocyclic compounds with potential inhibitory activities of menthionine synthase catalysed reaction

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ABSTRACT

Methionine synthase catalyses the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine, producing methionine and tetrahydrofolate. This enzyme has been linked to the pathogenesis of anemias, neurodegenerative disorders and cancer. The development of potent and specific inhibitors of methionine synthase could therefore be used to explore the enzyme as a target for pharmacological intervention and elucidate its role in these disorders. Benzimidazole derivatives have been shown to inhibit methionine synthase by competing with the substrate 5-methyltetrahydrofolate. The aim of this article was to design and synthesize a novel series of substituted benzimidazole derivatives and assesses their inhibitory activity against purified rat liver methionine synthase.

Cobalamin-dependent methionine synthase was isolated and purified from rat liver. The inhibitors were assessed using an *in vitro*, colourimetric enzyme assay, kinetic analysis of the most potent compound (*S*)-1-(5-nitro-1*H*-benzimidazole-2-yl)ethanamine using Lineweaver-Burk plots revealed characteristics of mixed inhibition with K_i value of 450 μM and percentage inhibition 64%.

The results of this study have led to the identification of this lead compound with good inhibitory activity that can inform the design of the next generation inhibitors of methionine synthase.

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INTRODUCTION

Cobalamin-dependant methionine synthase (MetS) is one of the transmethylase enzymes that utilizes cobalamin derivative methylcobalamin (methylcobalamin (CH_3-cobalamin I)) as a cofactor[1]. MetS catalyses the transfer of the methyl group from 5-methyltetrahydrofolate to homocysteine via the cobalamin cofactor (CH_3-cobalamin), which circulates between +1 and +3 oxidation states (Figure 1). Crystal structure of cobalamin-dependent MetS revealed that it is consisting of four functional binding domains. They are homocysteine

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binding domain, 5-methyltetrahydro-folate binding domain, the third domain binds cobalamin cofactor and the fourth domain is an allosteric cofactor *S*-adenosyl-methionine (S-AdoMet)[2]. The reaction products methionine and tetrahydrofolate are closely correlated to important biochemical reactions of the methylation of DNA, lipids, proteins, and polyamine[3,4].
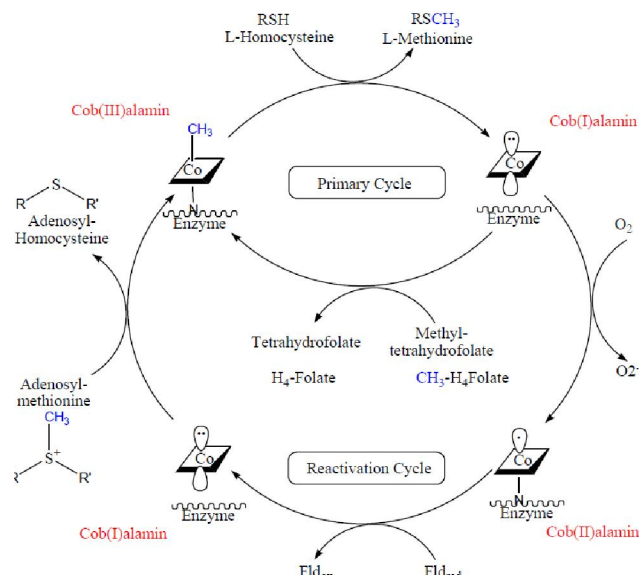

Figure 1 : Folic acid cycle

The present study showed a new approach for determining specific inhibitors of MetS. Benzimidazole derivatives that resembles substructure of 5-MTHF (Figure 2) and have been docked in to the MTHF binding domain. The free energy of binding of these ligand-receptor complexes have been obtained and compared to the results obtained from cell free assay.
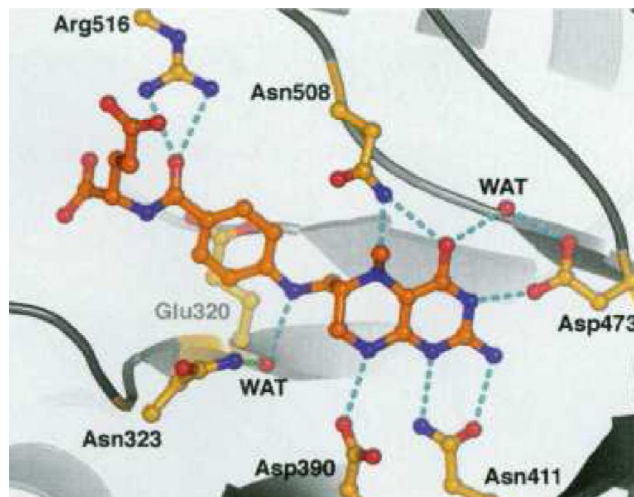

Figure 2 : Image of MTHF and its interactions with the conserved residues of the binding site. (Front View)

EXPERIMENTAL PROCEDURES

Molecular modeling procedure

Molecular modeling was carried out on Schrodinger computational software workstation using Maestro 9.1 graphic user interface (GUI) and Red Hat Linux nash Enterprise version V 4.1.18 on Batchmin V 9.1 modeling engine. The atomic coordinates for the polypeptide segments of MTHF and Hcy binding domains of MetS enzyme extracted from human liver were obtained from the protein bank database [PDB (Brookhaven protein database). Explicit calcium ion counter ions were included instead of cadmium and positioned at a 4Å distance from the homocysteine binding site. The basic and acidic amino acids were neutralized at PH 7 $\pm$ 2 by protonation of the terminal amino groups such as histidine, lysine and arginine. Moreover, the terminal carboxylic acid groups of amino acids and acidic amino acids such as glutamic acid, aspartic acid, asparagine and glutamine were deprotonated. Prime 1.5 was used to check the energy content of the protein segments and loops. The docking process involved the standard precision docking (SP) in which ligand poses that were expected to have unfavourable energies would be rejected. The presumption is that only active compounds will have available poses that avoid these penalties. SP docking is appropriate for screening ligands of unknown quality in large numbers. SP is a soft docking programme that was adept at identifying ligands that have a reasonable propensity to bind and 20 % of the final poses produced from the SP docking were subjected to the Extra Precision mode of Glide docking (XP) to perform the more expensive docking simulation on worthwhile poses. XP docking mode is harder than SP docking mode in that it penalizes the poses that violate established charges. Flexible docking was selected to generate conformations of all possible ligand poses, which is more realistic as this occurs in reality because the protein undergoes side chain and back bone movement or both, upon ligand binding. Five and six-membered rings were allowed to flip and amide bonds which were not *cis* or *trans* configuration were penalised. 5000 poses per ligand for the initial phase of docking with a scoring window for keeping poses of 100 KJ/mol were set up. The best poses which fulfil those conditions were subjected to energy minimization on the OPLS-AA non-

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bonded interaction grid with a distance dielectric constant of 2 and maximum number of conjugate gradient steps of 500 iterations. The ligands of the poses selected by the initial screening were subsequently minimized in the field of the receptor using a standard molecular mechanics energy function (OPLS-AA force field) in conjunction with a distance-dependant dielectric model. Finally, the lowest energy poses obtained in this fashion were subjected to a Monte Carlo procedure that examines nearby torsion minima. The complex was minimized using the conjugate gradients algorithm until an energy convergence criterion of 0.1 KJ/mol was reached with iteration cycle of 10,000. Molecular dynamics (MD) at 300 K were then performed on the solvated system for a 10 ps equilibration and 100 ps of production employing a 1 fs time step using OPLS-2005 force field, from which 100 structures were sampled at 1 ps intervals and averaged. The final averaged structure was then finally minimized[5].

Experimental procedure

General method for the synthesis of (1*H*-benzimidazole-2-yl)alkylamine compounds (Method A) General procedure for the synthesis of (1*H*-benzimidazole-2-yl)alkylamine compounds adapted from the Phillips procedure. A slight excess of L-amino acid was added to a magnetically stirred solution of 1,2-phenylenediamine and aqueous hydrochloric acid (5.5 M). The mixture was heated at reflux temperature until 1,2-phenylenediamine was no longer detectable by NMR or until consumption of the starting materials had ceased. The characteristically bright blue reaction mixture was cooled to room temperature. The mixture was allowed to stand for several weeks where upon the desired (1*H*-benzimidazole-2-yl)alkylamine was yielded in its dihydrochloride salt form. The resulting crystals were washed with acetone (3 x 20 ml) and recrystallised from water. Alternatively, the reaction mixture was concentrated to one third under reduced pressure and cooled to produce the desired benzimidazole as a dihydrochloride salt.

The free base was obtained by neutralisation of the reaction mixture with triethylamine followed by extraction of the pink solution with ethyl acetate. The extract was evaporated to dryness and recrystallised from an appropriate solvent. General procedure for the synthe-

sis of simple benzimidazole ring (Method B). General method for the synthesis of benzimidazole ring was based on the Phillips procedure[6].

An appropriate carboxylic acid was added to a stirring solution of a 1,2-phenylenediamine and aqueous hydrochloric acid (5.5 M). The mixture was heated at reflux temperature until the reaction had gone to completion. The reaction mixture was cooled to room temperature. The cooled reaction mixture was placed on ice and excess ammonia solution added to neutralise the solution. The pink/ grey precipitate that formed was isolated by filtration and recrystallised from a suitable solvent.
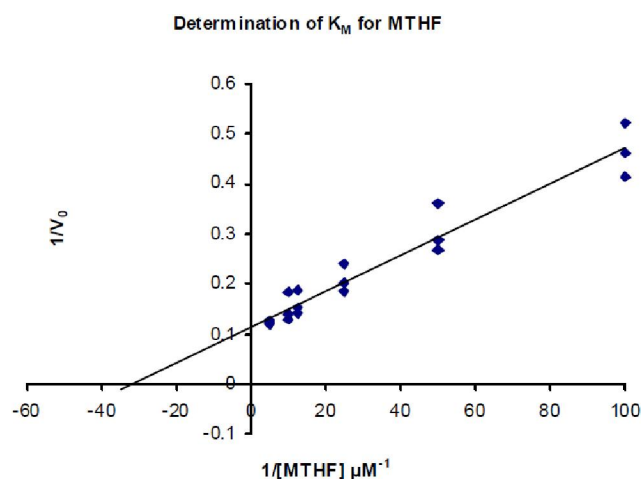

Figure 3 : Lineweaver-burk plot for MTHF, KM= 28.6 µM, Vmax= 8.7 µM min-1

(a) Synthesis of (1*H*-benzimidazole-2-yl)methylamine dihydrochloride 1

(1*H*-Benzimidazole-2-yl)methylamine dihydrochloride was prepared according to general procedure A, using 1,2-phenylenediamine (10.80 g, 0.10 mol) and glycine (12.65 g, 0.15 mol) dissolved in hydrochloric acid (70 ml, 5.5 M). The mixture was heated under reflux for 300 hrs. The reaction mixture was then cooled to room temperature and left to evaporate slowly over several days. The resulting blue crystals were washed with acetone. (1*H*-Benzimidazole-2-yl) methylamine dihydrochloride was obtained (13.30 g, 61%), m.p. 269-270 ºC.

δ_H (270 MHz; DMSO-d_6; Me_4Si) 7.61 (2H, dd, ^3J 9.40, ^4J 3.20, 4-H, 7-H), 7.27 (2H, m, ^3J 9.40, ^4J 3.20, 5-H, 6-H), 4.43 (2H, s, CH_2), 5.02 (3H, br, s, NH, NH_2); δ_C (68 MHz; DMSO-d_6; Me_4Si) 147.56 (C-2), 132.51 (C-3a, C-7a), 126.16 (C-5, C-6),

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114.99 (C-4, C-7), 35.17 (C-1'); DEPT (135) (DMSO-d$_6$); CH$_2$: 35.17, CH: 126.16, 114.99; υ_{max}/ cm^{-1} (KBr) 3050 (C-H, sp^2), 2850 (C-H, sp^3), 1450 and 1350 (C-H, bend); MS (EI): m/z 147 (M$^{+\cdot}$, 100 %), 119 (92, M$^{+\cdot}$- CHNH$_2$), 91 (17), 65 (10), 52 (4). Elemental analysis: Found; C, 44.66%; H, 5.04%; N, 19.09%; C$_8$H$_{11}$N$_3$.2HCl, Requires; C, 44.86%; H, 5.26%; N, 19.46%.
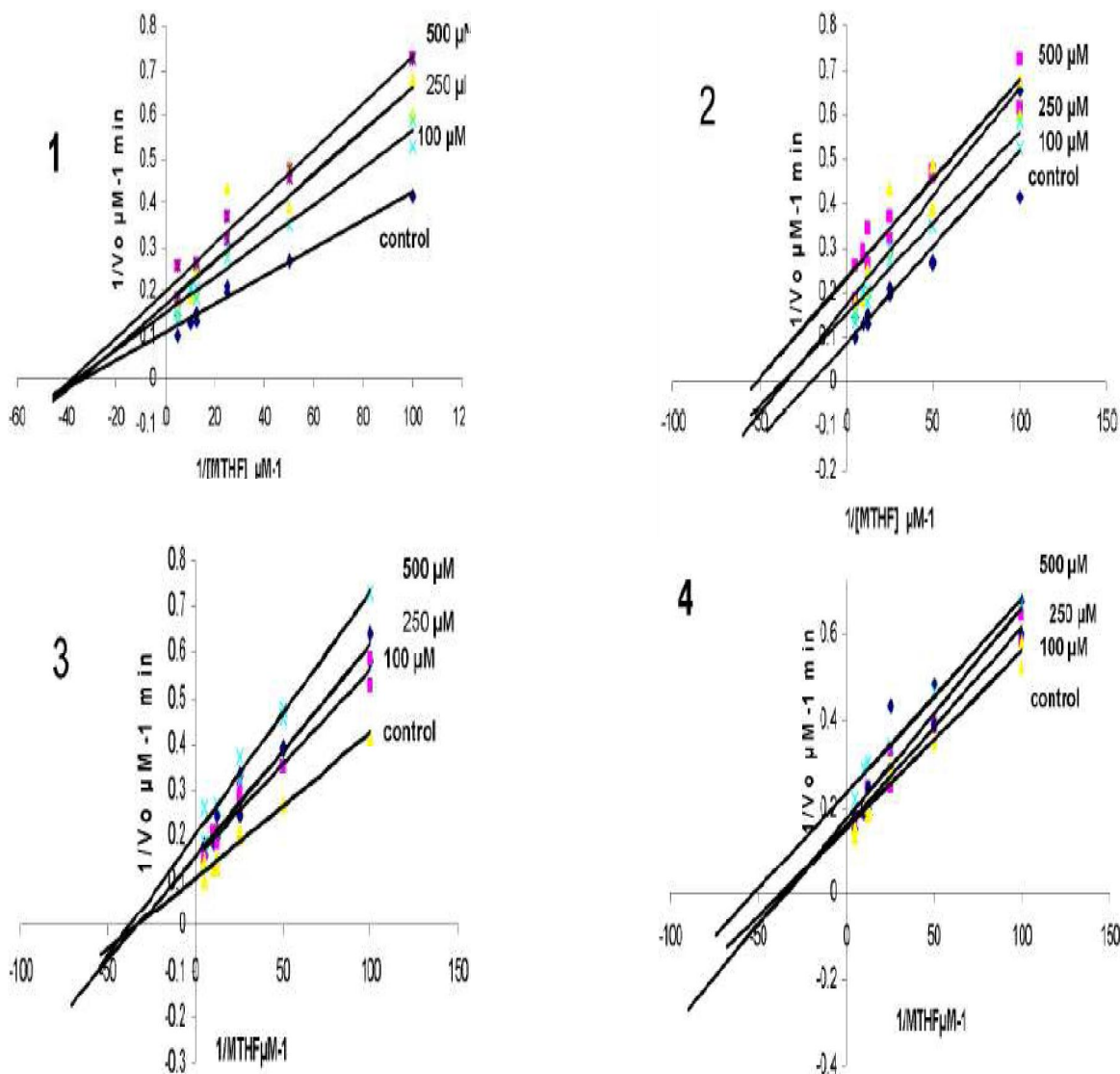

Figure 4 : Lineweaver-burk plot for compounds 1-4

(b) Synthesis of 2-(5-fluoro-1H-benzimidazole-2-yl)ethanamine dihydrochloride 2

2-(5-Fluoro-1H-benzimidazole-2-yl)ethanamine dihydrochloride was synthesized according to general method A using 4-fluoro-1,2-phenylenediamine (1.50 g, 12 mmol) and β-alanine (1.33 g, 15 mmol) dissolved in hydrochloric acid (30 ml, 5.5 M). The reaction was heated under reflux for 180 hrs. The resulting bright blue/ green reaction mixture was crystallised on cooling to give 2-(5-fluoro-1H-benzimidazole-2-yl)ethanamine (2.31 g, 76 %) in its dihydrochloride salt form, m.p. 294-296 °C.

δ_H (DMSO-d$_6$) 8.54 (1H, br, s, NH), 7.79 (1H, q, ^3J 8.90, ^4J 4.40, 7-H), 7.63 (1H, dd, ^3J 8.70, ^4J 2.50, 4-H), 7.35 (1H, td, ^3J 5.00, ^4J 2.50, 6-H), 3.55 (2H, t, ^3J 5.69, NH_2), 2.95 (2H, q, ^3J 6.93, ^3J 5.69, 2'-H), 2.63 (2H, t, ^3J 6.93, 1'-H); δ_C (DMSO-d$_6$) 162.1 (C-5), 152.20 (C-2), 132.5 (C-3a), 128.7 (C-7a), 115.9 (C-7), 114.1 (C-6), 101.2 (C-4), 36.4 (C-2'), 25.4 (C-1'); υ_{max}/cm^{-1} 3438 (N-H), 3050 (C-H, sp^2), 2850 (C-H, sp^3), 1140 (C-F); MS (EI): m/z 179 (M$^{+\cdot}$, 100%), 150 (79, M$^{+\cdot}$-CHNH$_2$), 91(12), 65(9);

Elemental analysis: Found; C, 42.83%; H, 4.98%;

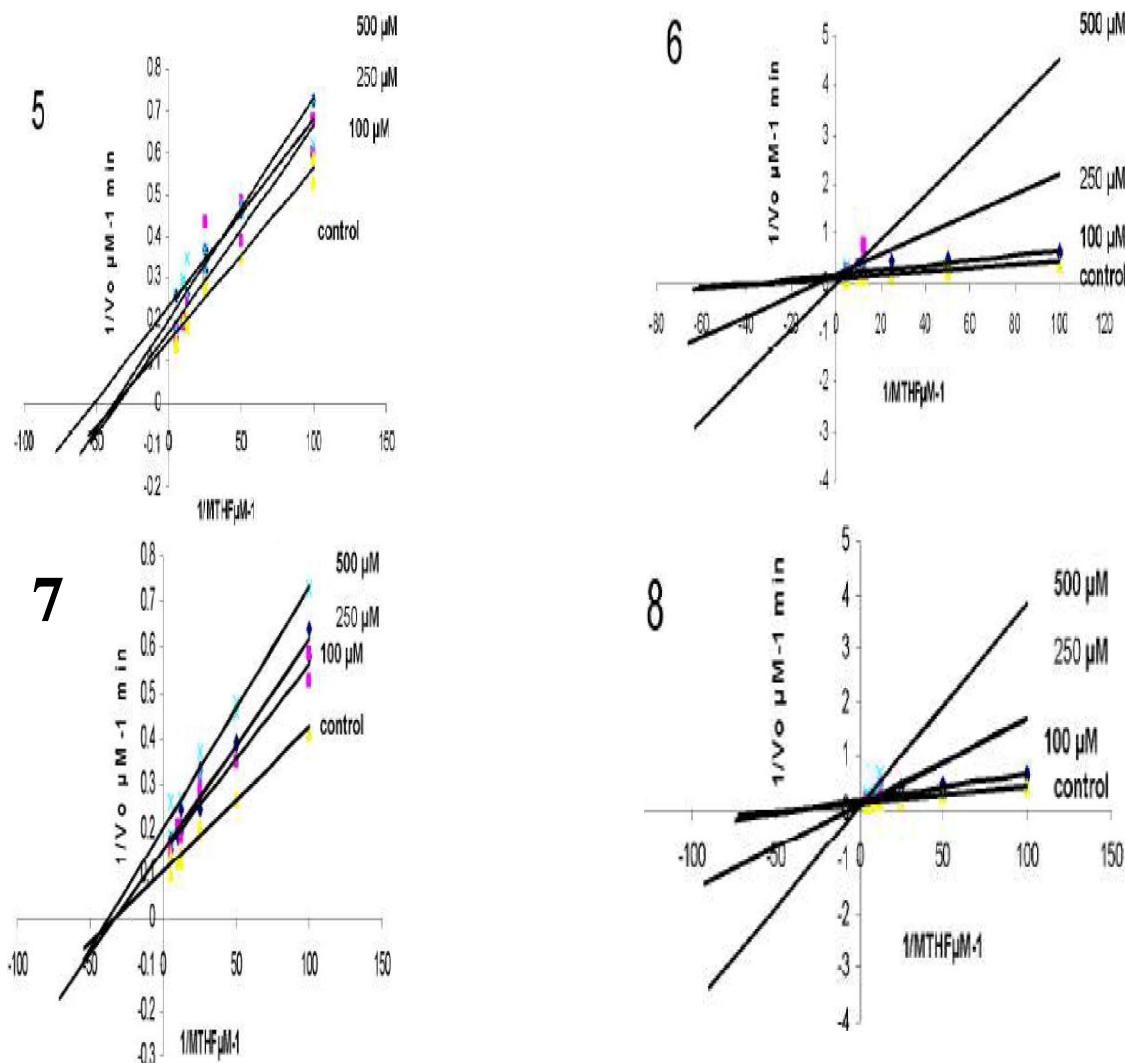N, 16.56%; $C_9H_{10}N_3F.2HCl$, Requires; C, 42.86%; H, 4.76%; N, 16.66%.

Figure 5 : Lineweaver-burk plot for compounds 5-8

(c) Synthesis of 2-(5-chloro-1*H*-benzimidazole-2-yl)ethanamine dihydrochloride 3

2-(5-Chloro-1*H*-benzimidazole-2-yl)ethanamine dihydrochloride was prepared according to general method A, using 4-chloro-1,2-phenylenediamine (3.56 g, 25 mmol), and β-alanine (2.50 g, 28 mmol) dissolved in hydrochloric acid (40 ml, 5.5 M). The reaction was heated at reflux temperature for 240 hrs. The resulting dark brown / red reaction mixture was filtered before leaving to evaporate slowly. 2-(5-Chloro-1*H*-benzimi-dazole-2-yl)ethanamine dihydrochloride (5.01 g, 75%) was recovered as a pale green solid, m.p. decomp. 282-285 °C.

(Cherkaoui et al, 1991) δ_H(D$_2$O) 7.73 (1H, d, ^4J 2.00, 4-H), 7.62 (1H, d, ^3J 8.70, 7-H), 7.46 (1H, dd,

^3J 8.90, ^4J 2.00, 6-H), 3.58 (4H, m, 1'-H, 2'-H, C*H*C*H$_2$*), NHs not observed; δ_C (D$_2$O) 149.8 (C-2), 131.8 (C-3a), 131.4 (C-7a), 129.6 (C-5), 127.1 (C-6), 115.2 (C-7), 113.9 (C-4), 36.6 (C-2'), 24.6 (C-1'); υ_{max} /cm^{-1} (KBr) 3425 (N-H), 3012 (C-H, sp^2), 2850 (C-H, sp^3), 750 (C-Cl); MS (ESI+): m/z 196 (M$^+$+1, 100%), 116.1 (21) 108.2 (4), 87.0 (6). Elemental analysis: Found; C, 42.83%; H, 4.98%; N, 15.66%; $C_9H_{12}Cl_3N_3$, Requires; C, 42.25%; H, 4.50%; N, 15.65%.

(d) Synthesis of 2-(5-methyl-1*H*-benzimidazole-2-yl)ethanamine dihydrochloride 4

2-(5-Methyl-1*H*-benzimidazole-2-yl)ethanamine dihydrochloride (Muftic, 1969, Lahr and Muftic, 1967) was prepared according to general method A, using 4-

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methyl-1,2-phenylenediamine (6.24 g, 51.14 mmol) and β-alanine (5.35 g, 61.23 mmol) in hydrochloric acid (55 cm, 5.5 M). The mixture was heated at reflux for 80 hrs. The dark blue reaction mixture yielded fine blue needles of 2-(5-methyl-1H-benzimidazole-2-yl)ethanamine dihydrochloride (11.2 g, 88%) on cooling, m.p. decomp. 300-302 °C, δ_H(D$_2$O) 7.57 (1H, d, ^3J 8.40, 7-H), 7.52 (1H, d, 4-H, ^4J 1.20), 7.40 (1H,

dd, ^3J 8.40, ^4J 1.20, 6-H), 3.54-3.54 (4H, m, CH_2CH_2), 2.45 (3H, s, CH_3), NHs not observed; (DMSO-d$_6$) 150.2 (C-2), 135.9 (C-3a), 131.9 (C-7a), 129.7 (C-5), 127.5 (C-6), 113.9 (C-4), 113.8 (C-7), 36.3 (C-2'), 25.2 (C-1'), 21.7 (CH_3), Elemental analysis: Found; C, 48.03%; H, 6.52%; N, 16.36%; C$_{10}$H$_{15}$Cl$_2$N$_3$ Requires; C, 48.40%; H, 6.09%; N, 16.93%.
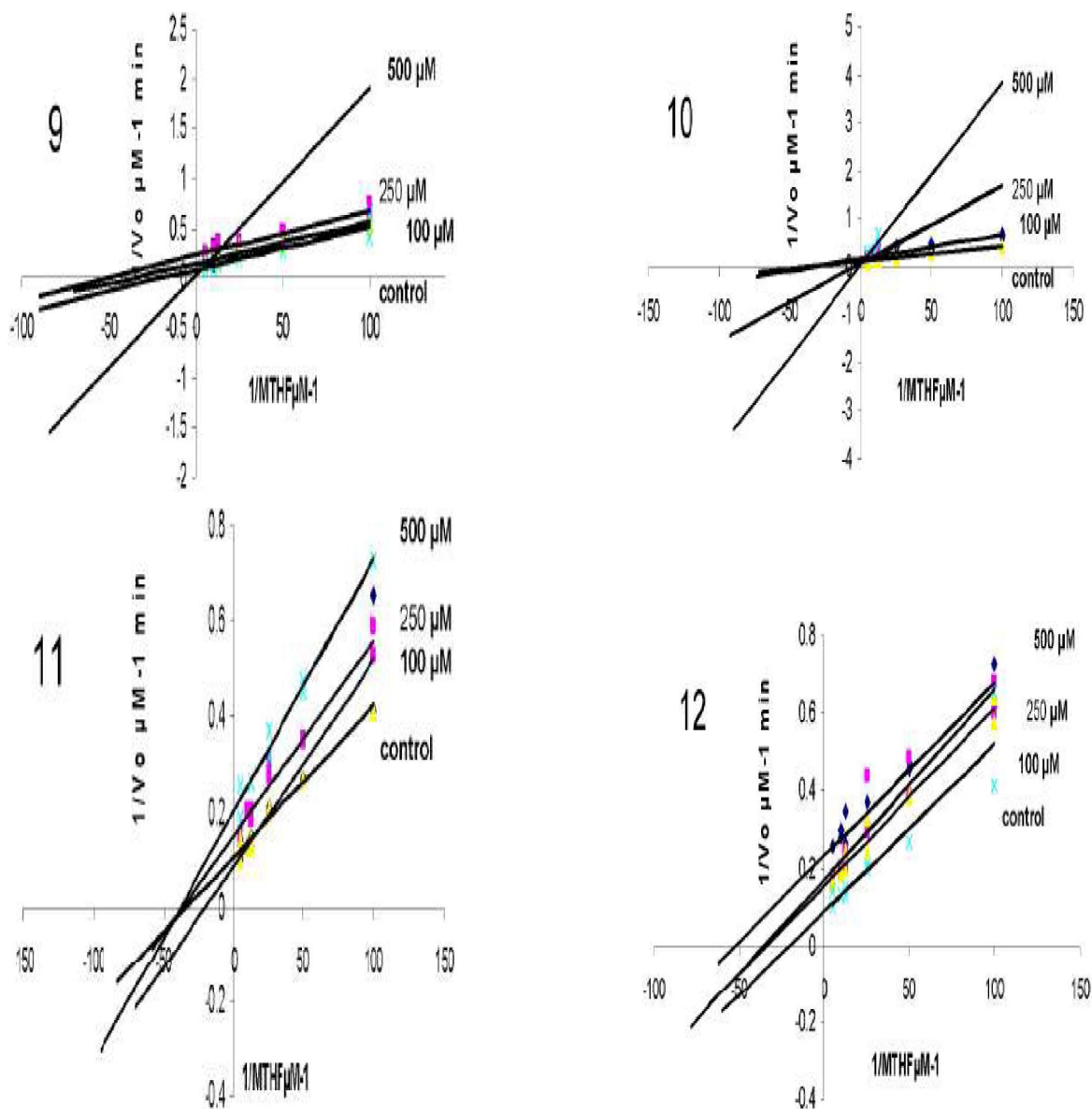

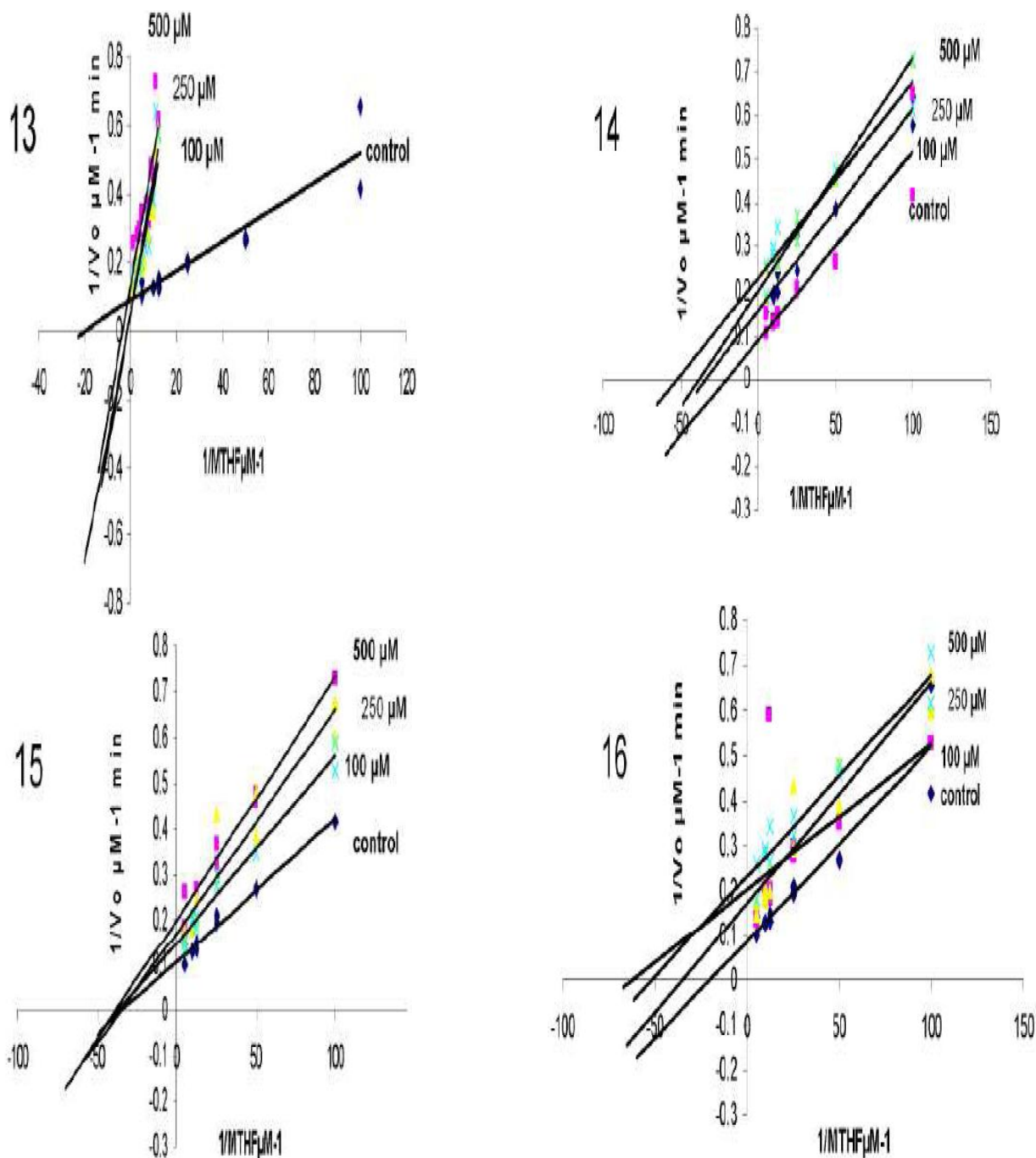**Figure 6 : Lineweaver-burk plot for compounds 6-12**

(e) Synthesis of (*S*)-1-(1*H*-benzimidazole-2-yl)ethanamine 5

(*S*)-1-(1*H*-benzimidazole-2-yl)ethylamine was prepared according to general procedure A using 1,2-phenylenediamine (11.00 g, 0.102 mol) and L-alanine (16.00

g, 0.18 mol) in hydrochloric acid (70 ml; 5.5 M). The mixture was heated at reflux for 144 hrs after which time a further portion of 1,2-phenylenediamine (5.00 g, 0.046 mol) was added and refluxing continued for a further 120 hrs. The product contained 1,2-phenylene-

diamine (12%). (*S*)-1-(1*H*-Benzimidazole-2-yl)ethanamine (1.32 g, 8%) was obtained as a red solid, m.p. 296-298 °C. δ_H (DMSO-d$_6$) 7.50 (2H, dd, ^3J 5.93, ^4J 3.2, 4-H, 7-H), 7.13 (2H, dd, ^3J 5.93, ^4J 3.2, 5-H, 6-H), 4.19 (1H, q, ^3J 6.92, C*H*), 1.44 (3H, d, ^3J 6.92, C*H*$_3$), NHs not observed. Elemental analysis: Found; C, 67.43%; H, 6.98%; N, 26.46%; C$_9$H$_{11}$N$_3$, Requires; C, 67.06%; H, 6.88%; N, 26.07%.

Figure 7 : Lineweaver-burk plot for compounds 13-16

(f) Synthesis of (*S*)-1-(5-nitro-1*H*-benzimidazole-2-yl)ethanamine 6

(*S*)-1-(5-Nitro-1*H*-benzimidazole-2-yl)ethanamine was prepared according to general procedure A using 4-nitro-1,2-phenylenediamine (3.06 g, 20 mmol) and L-alanine (2.00 g, 22.5 mmol) in hydrochloric acid (60 ml, 5.5 M). The mixture was heated at reflux for 336 hrs. The reaction mixture was evaporated to dryness and dissolved in minimum ammonia. Water was added and the product extracted with ethyl acetate. (*S*)-1-(5-
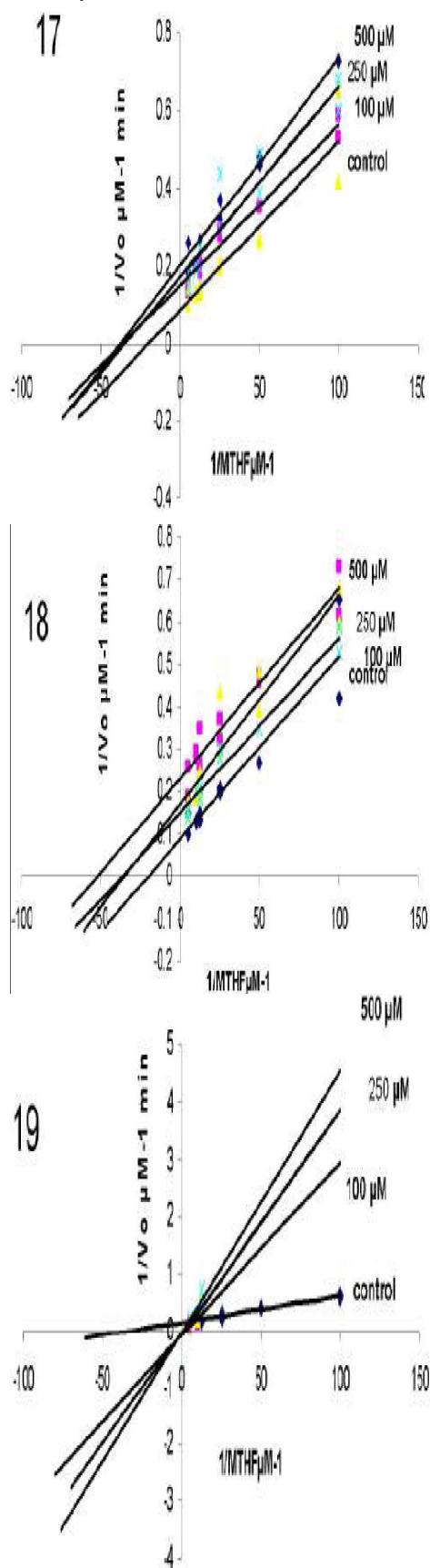
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Figure 8 : Lineweaver-burk plot for compounds 17-19

Nitro-1*H*-benzimidazole-2-yl)ethanamine (4.03 g) containing 4-nitro-1,2-phenylenediamine (50%) and alanine (25%) was recovered.

δ_H (DMSO-d$_6$) 8.62 (1H, d, 4-H), 8.56 (1H, dd, ^3J 8.40, 6-H), 8.38 (1H, d, ^3J 8.40, 7-H), 4.26 (1H, q, ^3J 6.70, 1'-H), 1.45 (3H, d, ^3J 6.70, 2'-H), Elemental analysis: Found; C, 52.83%; H, 4.98%; N, 27.56%; $C_9H_{10}N_4O_2$, Requires; C, 52.42%; H, 4.89%; N, 27.17%.

(g) Synthesis of (*S*)-1-(5-fluoro-1*H*-benzimidazole-2-yl)ethanamine dihydrochloride 7

(*S*)-1-(5-Fluoro-1*H*-benzimidazole-2-yl)ethanamine dihydrochloride was prepared according to general method A, using 4-fluoro-1,2-phenylenediamine (0.15 g, 1.2 mmol), L-alanine (0.11 g, 1.23 mmol) and hydrochloric acid (20 ml, 5.5 M). The mixture was heated at reflux for 288 hrs. The resulting green solution was evaporated to dryness and recrystallized from ethanol to obtain the product as green/brown oil.

δ_H (DMSO-d$_6$) 7.56 (1H, d, ^3J 8.80, 7-H), 7.35 (1H, d, 4-H), 7.00 (1H, dd, ^3J 8.80, 6-H), 4.18 (1H, q, ^3J 6.90, 1'-H), 1.40 (3H, d, ^3J 6.90, 2'); MS (EI): m/z 179.1 (M$^+$, 43%), 164.1 (60, M$^+$-CH$_3$), 136.1 (51, M$^+$-CH$_3$CHNH), 77.0 (14), Elemental analysis: Found; C, 42.83%; H, 4.88%; N, 16.26%; $C_9H_{12}Cl_2FN_3$, Requires; C, 42.88%; H, 4.80%; N, 16.67%.

(h) Synthesis of (*S*)-1-(5-chloro-1*H*-benzimidazole-2-yl)ethanamine 8

(*S*)-1-(5-Chloro-1*H*-benzimidazole-2-yl)ethanamine was prepared according to general method A, using 4-chloro-1,2-phenylenediamine (0.15 g, 1.2 mmol), L-alanine (0.11 g, 1.23 mmol) and hydrochloric acid (20 ml, 5.5 M). The mixture was heated at reflux for 288 hrs. The resulting dark brown solution was evaporated to dryness. (*S*)-1-(5-Chloro-1*H*-benzimidazole-2-yl)ethanamine (0.05 g, 21.3%) was obtained as a dark brown solid m.p. 152-154 °C. δ_H (DMSO-d$_6$) 7.80-7.52 (2H, m, 4-H, 7-H), 7.23 (1H, m, 6-H), 4.25 (1H, q, ^3J 6.90, 1'-H), 1.43 (3H, d, ^3J 6.70, 2'-H); δ_C (DMSO-d$_6$) 161.7 (C-2), 140.7 (C-3a), 138.9 (C-7a), 126.1 (C-5), 122.6 (C-6), 116.3 (C-7), 115.2 (C-4), 46.3 (C-1'), 15.2 (C-2'); MS (EI): m/z 244.3 (15), 165.1 (12), 115.1 (2), 77.0 (3); υ_{max}/cm^{-1} 3050 (C-H, sp^2), 2850 (C-H, sp^2), 1622 and

1445 (C=C); 814 (C-Cl), Elemental analysis: Found; C, 55.63%; H, 5.08%; N, 21.06%; $C_9H_{10}ClN_3$, Requires; C, 55.25%; H, 5.15%; N, 21.48%.
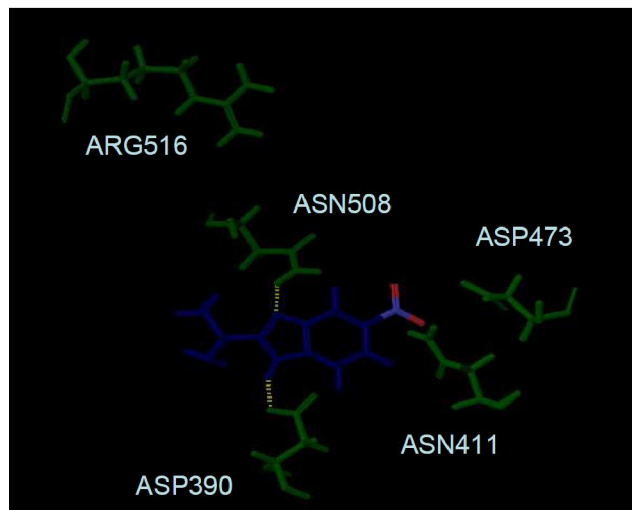

Figure 9 : Image of compound 6 docked in to the MTHF binding domain of MetS. (Front View)

(i) Synthesis of (*S*)-1(1*H*-benzimidazole-2-yl)-2-methylpropan-1-amine dihydrochloride 9

The synthesis of (*S*)-1(1*H*-benzimidazole-2-yl)-*2-methylpropan*-1-amine dihydrochloride was attempted according to general method A, from 1,2-phenylenediamine (1.78 g,) and L-valine dissolved in hydrochloric acid (50 ml, 5.5 M). The mixture was heated at reflux for 300 hrs. The resulting dark green reaction mixture yielded green crystals of 1,2-phenylenediamine and clear needles of 1,2-phenylenediamine as the hydrochloride salt. m.p. 162-164°C

δ_H (DMSO-d$_6$) 7.80 (2H, dd, ^3J 3.4, ^4J 3.2, 5-H, 6-H), 7.20 (2H, dd, ^3J 3.4, ^4J 3.2, 4-H, 7-H), 3.20 (1H, d, ^3J 1.2, 1'-H), 2.22 (1H, m, CH), 2.01(2H, brs, NH$_2$),1.20 (6H, d, ^3J 2.40, CH$_3$); δ_C (DMSO-d$_6$) 160.07 (C-2), 141.22 (C-3a), 138.01 (C-7a), 124.01 (C-5), 120.03 (C-6), 119.02 (C-7), 117.01 (C-4), 42.03 (C-1'), 16.12 (CH), 15.12 (CH$_3$); MS (EI): m/z 190 (44), 165.1 (22), 115.1 (12), 77.0 (13), Elemental analysis: Found; C, 50.00%; H, 6.08%; N, 16.02%; $C_{11}H_{17}Cl_2N_3$, Requires; C, 50.39%; H, 6.54%; N, 16.06%.

(j) Synthesis of (*S*)-1-(1*H*-benzimidazole-2-yl)-*3-methylbutan-1-amine dihydrochloride 10

Synthesis of (*S*)-1-(1*H*-benzimidazole-2-yl)-3-methylbutan-1-amine dihydrochloride was attempted

using general method A, from 1,2-phenylenediamine (10.80 g, 0.1 M), L-leucine (16.00, 0.12 g) and hydrochloric acid (70 ml, 5.5 M). The mixture was heated at reflux for 300 hrs. The resulting dark green reaction mixture yielded green crystals of 1,2-phenylenediamine and clear needles of 1,2-phenylenediamine as the hydrochloride salt. m.p. 142-144°C.

δ_H (DMSO-d$_6$) 7.92 (2H, dd, ^3J 5.93, ^4J 3.2, 4-H, 7-H), 7.73 (2H, dd, ^3J 5.93, ^4J 3.2, 5-H, 6-H), 3.09 (1H, t, ^3J 5.02, 1'-H), 1.55 (2H, t, ^3J 5.02, CH$_2$), 1.00 (1H, m, CH), 0.94 (3H, d, ^3J 3.92, C*H*$_3$), NH not observed., Elemental analysis: Found; C, 52.73%; H, 4.98%; N, 16.56%; $C_{12}H_{19}Cl_2N_3$, Requires; C, 52.18%; H, 5.03%; N, 16.01%.

(k) Synthesis of (*S*)-2-amino-4- (1*H*-benzimidazole-2-yl) butyric acid dihydrochloride 11

(*S*)-2-Amino-4-(1*H*-benzimidazole-2-yl) butyric acid was prepared according to general procedure A. 1,2-Phenylenediamine (11.00 g, 0.102 mol) and L-glutamic acid (20.00 g, 0.14 mol) were heated at refluxing temperature for 145 hrs until no traces of 1,2-phenylenediamine could be detected by NMR. The dark green reaction mixture was evaporated to dryness resulting in the crude product as a green paste (16.98 g, 76%), m.p. 298-300 °C. δ_H (DMSO-d$_6$) 8.85 (1H, br, s, NH), 8.40 (2H, br, s, N*H*$_2$), 7.79 (2H, dd, ^3J 8.66, ^4J 2.96, 4-H, 7-H), 7.53 (2H, dd, ^3J 8.90, ^4J 2.96, 5-H, 6-H), 4.11 (1H, t, 3'-H), 3.52 (2H, t, 1'-H), 3.38 (2H, q, 2'H); MS (EI): m/z 219,1 (M$^{+\cdot}$, 2.0 %), 145.1 (100), 132.1 (53.0), 84.0 (24.5), 65.0 (11.5), Elemental analysis: Found; C, 45.45%; H, 5.80%; N, 14.56%; $C_{11}H_{15}Cl_2N_3$, Requires; C, 45.22%; H, 5.17%; N, 14.38%.

(l) Synthesis of (1*H*-benzimidazole-2-yl)methanol 12

(1*H*-Benzimidazole-2-yl)methanol was prepared according to general method B. 1,2-Phenylenediamine (10.80 g, 0.10 mol) and glycolic acid 50% (30 ml) were dissolved in hydrochloric acid (70 ml, 5.5 M) and the mixture heated under reflux for 3 h. The reaction mixture was neutralised with ammonia solution and the pink precipitate recrystallised from water. (1*H*-Benzimidazole-2-yl)methanol (10.4 g, 71%), was obtained as pure while crystals, m.p. 170-171 °C. δ_H (DMSO-d$_6$) 7.50 (2H, dd, ^3J 9.15, ^4J 3.46, 4-H, 7-H), 7.14 (2H, dd, ^3J

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9.15, ^4J 3.46, 5-H, 6-H), 5.74 (1H, br, s, O*H*, exchanged with D_2O), 4.70 (2H, s, CH_2); υ_{max}/cm^{-1} (KBr) 3300- 3200 (O-H), 3050 (C-H, sp^2), 2850 (C-H, sp^3), 1450 and 1350 (C-H, bend); MS (EI): m/z 148 (M$^{+\cdot}$, 100%), 130 (44 M-H_2O), 119 (49, M-CHO), 90 (20.1), 64 (16.2), Elemental analysis: Found; C, 64.43%; H, 5.98%; N, 18.66%; $C_8H_8N_2O$, Requires; C, 64.85%; H, 5.44%; N, 18.91%.

(m) Synthesis of 5-chloro-1*H*-benzimidazole-2-yl)methanol 13

5-Chloro-1*H*-benzimidazole-2-yl)methanol was prepared according to general method B, using 4-chloro-1,2-phenylenediamine (2.00 g, 14 mmol), glycolic acid (1.22 g, 16 mmol) and hydrochloric acid (40 ml, 5.5 M). The solution was heated at reflux for 8 hrs. The crude product was dissolved in ethanol and decolourised with activated charcoal prior to recrystallisation. 5-Chloro-1*H*-benzimidazole-2-yl)methanol (1.64 g, 64%) was recovered as brown/red coloured crystals, m.p. 207- 208 °C. δ_H (DMSO-d_6) 7.54 (1H, d, ^4J 2.00, 4-H), 7.50 (1H, d, ^3J 8.70, 7-H), 7.15 (1H, dd, ^3J, 8.70, ^4J 2.00, 6-H), 5.80 (1H, br, s, O*H*, exchanged with D_2O), 4.89 (2H, s, CH_2), N*H* not observed; δ_C (DMSO-d_6) 157.25 (C-2), 144.10 (C-3a), 136.00 (C-7a), 126.22 (C-5), 122.13 (C-6), 118.40 (C-7), 111.57 (C-4), 58.21 (C-1'); υ_{max}/cm^{-1} (KBr) 3135 (O-H), 3050 (C-H, sp^2), 2820 (C-H, sp^3), 700 (C-Cl); MS (ESI+): m/z 183.0 (M^{+}+1, 100%), 165.0 (18, M$^{+\cdot}$-OH), 145.0 (5); Elemental analysis: Found; C, 52.81%; H, 3.75%; N, 15.21%; $C_8H_7N_2OCl$; Requires; C, 52.60%; H, 3.83%; N, 15.34%.

(n) Synthesis of (5-fluoro-1*H*-benzimidazole-2-yl)methanol 14

(5-Fluoro-1*H*-benzimidazole-2-yl)methanol was synthesized according to general method B using, 4-fluoro-1,2-phenylenediamine (1.26 g, 10 mmol) and glycolic acid (0.80 g, 10.5 mmol) dissolved in hydrochloric acid (30 ml, 5.5 M). The mixture was heated under reflux for 8 hrs. The brown crude product was decolourised and recrystallised from ethanol to give 5-fluoro-1*H*-benzimidazole-2-yl)methanol (1.02 g, 62%) as pale cream crystals, m.p. 182-183 °C. δ_H (DMSO-d_6) 7.47 (1H, m, ^3J 8.40, ^4J 5.00, 7-H), 7.26 (1H, d, ^3J 9.40, 4-H), 6.97 (1H, m, ^3J 8.40, 6-H); 5.80 (1H,

br, s, OH, exchanged with D_2O), 4.67 (2H, s, 1'-H), N*H* not observed; δ_C (DMSO-d_6) 160.5 (C-2), 157.1 (C-5), 139.6 (C-3a), 135.5 (C-7a), 115.7 (C-7), 110.5 (C-6), 101.5 (C-4), 58.2 (C-1'); υ_{max}/cm^{-1} (KBr) 3500 (O-H), 3100 (C-H, sp^2), 2850 (C-H, sp^3), 1634 and 1505 (C=C),1462 (C-H, bend), 1332 (C-F); MS (EI): m/z 166.0 (M$^{+\cdot}$, 100%), 149.0 (39, M$^{+\cdot}$-OH), 137.0 (59, CHO), 121.0 (40), 109.0 (32); 77.0 (5), 63.0 (7); Elemental analysis: Found; C, 57.94%; H, 3.94%; N, 16.48%; $C_8H_7N_2OF$, Requires; C, 57.83%; H, 4.22%; N, 16.87%.

(o) Synthesis of (5-methyl-1*H*-benzimidazole-2-yl)methanol 15

(5-Methyl-1 H-benzimidazole-2-yl)methanol was prepared according to method B. 4-Methyl,1,2-phenylenediamine (12.22 g, 0.1 mol) was heated at reflux temperature with glycolic acid (11.40 g, 0.15 mmol) in hydrochloric acid (50 ml, 5.5 M) for 3 hrs. The reaction mixture was cooled to room temperature and ammonia solution was added and the mixture cooled in ice until a pale pink/ grey precipitate formed. The resulting solid was recrystallised from aqueous ethanol to give a pale creamy yellow powdery solid. (5-Methyl-1 H-benzimidazole-2-yl)methanol (13.7 g, 85%) was recovered, m.p. 202-203 ºC.

δ_H (DMSO-d_6) 7.40 (1H, d, ^3J 8.16, 7-H), 7.30 (1H, d, 4-H), 7.00 (1H, dd, ^3J 8.16, 6-H), 4.70 (2H, s, CH_2), 2.40 (3H, s, CH_3), OH not observed; MS (EI): m/z 162 (M$^{+\cdot}$, 100 %), 133 (60, M$^{+\cdot}$-CH_2OH), 104 (38), 77 (40), 51 (19), Elemental analysis: Found; C, 67.02%; H, 6.18%; N, 17.56%; $C_9H_{10}N_2O$, Requires; C, 66.65%; H, 6.21%; N, 17.27%.

(p) Synthesis of (1*H*-benzothiazol-2-yl)methanol 16

Glycolic acid 99% (11.40 g, 0.15 mol) was dissolved in 2-aminothiophenol (12.5 g, 0.1 mol) in the absence of a solvent. The viscous solution was heated at reflux temperature for 5 hrs. The reaction mixture was cooled to room temperature before adding ethanol (30 ml). Water (100 ml) was then added to the bright yellow mixture and the resulting oil isolated. On standing the oil solidified and was subsequently crystallised from aqueous ethanol. (1*H*-Benzothiazol-2-yl)methanol was isolated as bright yellow needles (15.0 g, 91%), m.p. 101-102°C.

δ_H (DMSO) 8.10 (1H, dd, ^3J 7.91, ^4J 1.48, ^5J 0.74,

4-H), 7.92 (1H, q, ^3J 7.91, ^4J 1.48, ^5J 0.74, 7-H), 7.43 (2H, m, ^3J 7.91, ^4J 1.48, 5-H, 6-H), 6.27 (1H, t, ^3J 5.94, O*H*), 4.86 (2H, d, ^3J 5.94, C*H$_2$*); MS (EI): (M$^{+\cdot}$, 42%), 136.1 (100, M$^{+\cdot}$- CHO), 108 (35), Elemental analysis: Found; C, 58.40%; H, 4.77%; N, 8.50%; C$_8$H$_7$NOS, Requires; C, 58.16%; H, 4.27%; N, 8.48%.

(q) Synthesis of 2-methylbenzimidazole 17

2-Methylbenzimidazole was prepared according to general method B using 1,2-phenylenediamine (10.80 g, 0.1 mol), acetic anhydride (12.00 g, 0.12 mol) and hydrochloric acid (70 ml, 5.5 M). The reaction was heated at reflux temperature for 3 hrs and neutralised with ammonia solution on completion. The crude product was recrystallised from aqueous ethanol to give creamy-white coloured crystals of 2-methylbenzimidazole (9.10 g, 69%), m.p. 176- 177 °C.

δ_H (DMSO-d$_6$) 7.45 (2H, dd, ^3J 5.94, ^4J 3.21, 4-H, 7-H), 7.09 (2H, dd, ^3J 5.94, ^4J 3.21, 5-H, 6-H), 2.48 (3H, s, C*H$_3$*), Elemental analysis: Found; C, 73.03%; H, 6.02%; N, 21.56%; C$_8$H$_8$N$_2$, Requires; C, 72.86%; H, 6.10%; N, 21.20%.

(r) Synthesis of 2-methyl-5-nitro-1*H*-benzimidazole 18

4-Nitro-1,2-phenylenediamine (7.65 g, 0.05 mol) and acetic anhydride (6.00 g, 0.06 mol) were heated at reflux in the presence of hydrochloric acid (30 ml, 5.5 M) for 8 hrs. The reaction mixture was cooled to room temperature before neutralisation with ammonia hydroxide solution. The resulting precipitate on cooling the neutralised solution in ice was isolated. The crude product was decolourised with charcoal and recrystallised from aqueous ethanol. 2-Methyl-5-nitro-1*H*-benzimidazole (5.31 g, 60%) was recovered as cream crystals, m.p. 221-222 °C.

δ_H (DMSO-d$_6$) 8.33 (1H, dd, ^4J 1.80, ^5J 1.50, 4-H), 8.04 (1H, dd, ^3J 8.90, ^4J 2.20, 6-H), 7.60 (1H, dd, ^3J 8.90, ^5J 1.50, 7-H), 2.55 (3H, s, C*H$_3$*), Elemental analysis: Found; C, 54.03%; H, 4.08%; N, 23.16%; C$_8$H$_7$N$_3$O$_2$, Requires; C, 54.24%; H, 3.98%; N, 23.72%.

(s) Synthesis of 5-methylbenzimidazole 19

5-Methylbenzimidazole was prepared according to general method B. 4-Methyl-1,2-phenylenediamine

(4.20 g, 35 mmol) was dissolved in formic acid (2.30 g, 50 mmol) and the mixture heated at reflux for 4 hrs. 5-Methylbenzimidazole was obtained as a cream powder (4.08 g, 88%), m.p. 117-118 °C.

δ_H (DMSO-d$_6$) 7.32 (1H, d, ^3J 8.90, 7-H), 7.28 (1H, d, ^4J 2.22, 4-H), 7.21 (1H, s, 2-H), 6.81 (1H, dd, ^3J 8.90, ^4J 2.22, 6-H), 3.37 (3H, s, CH$_3$), NH not observed; δ_C (DMSO-d$_6$) 165.3 (C-2), 154.8 (C-3a), 147.4 (C-7a), 132.7 (C-5), 118.6 (C-6), 113.4 (C-4), 106.1 (C-7), 56.1 (CH$_3$), Elemental analysis: Found; C, 73.03%; H, 6.18%; N, 22.06%; C$_8$H$_8$N$_2$F, Requires; C, 72.70%; H, 6.10%; N, 21.20%.

BIOLOGICAL EVALUATION

Enzyme purification

Methionine synthase was isolated and purified from rat liver[7]. Anion exchange chromatography using DEAE and Q-Sepharose resins was used to purify methionine synthase. Anion exchange resins interact with proteins through electrostatic attractions. Bound proteins are eluted by increasing the ionic strength of the buffer. DEAE resins carry a positive charge on a tertiary amine, they are weak anion exchangers and have a low binding capacity. Q-Sepharose resins carry a positive charge on a quaternary amine, they are strong anion exchangers, and have a high protein binding capacity. The rat liver homogenate was prepared in the presence of a cocktail of protease inhibitors. These succeeded in inactivating proteolytic enzymes that could hydrolyse cytosolic proteins including methionine synthase. The cytosolic fraction was obtained following a series of centrifugation steps. This preparation was initially purified using batch chromatography on DEAE cellulose. Batch chromatography was employed due to the large quantities to be purified.

Batch chromatography is a convenient way to quickly partially purify large volumes of proteins. However, it does not have the versatility of column chromatography. The speedy removal of unwanted proteins in the unbound fraction enriched the enzyme preparation and facilitated the next step of the purification which was performed using column chromatography. The filtrate that was obtained from the DEAE cellulose batch chromatography procedure had a high salt concentra-

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tion. A three fold dilution of the filtrate was required to reduce this and allow binding of the proteins to the Q-Sepharose resin. A chromatogram of a typical separation is given (Figure 12). The broad peak on the left of the chromatogram shows the unbound fraction. During the loading of the column proteins that are unable to bind to the resin are washed through. The bound proteins were eluted at 2 ml/min and fractions collected at selected intervals throughout the run. Fractions collection commenced at the beginning of each peak until it tailed off. The red line indicates the salt gradient. The gradient was held where necessary to ensure that the peaks were sufficiently separated. The active fractions, shown by the blue bars were identified using the *in vitro* methionine synthase assay. The active fractions were pooled, desalted and concentrated. Two fractions, typically 100 ml in total were collected for each of the three recorded peaks. The 30 kDa membrane used for concentration of the enzyme preparation allowed salt, water and small proteins to pass though leaving a concentrated mixture of larger proteins including methionine synthase. The specific activity of the enzyme preparation was calculated to be 19.8 nmol methionine/ h/ mg of protein. A time-dependent assay was used to identify the time range in which the linear reaction occurred using 0.74 mg of protein per assay. The plot from this experiment is shown by Figure 13. The assays were performed in duplicate and results are expressed as individual values. The results show that the parameters investigated are within the linear reaction.
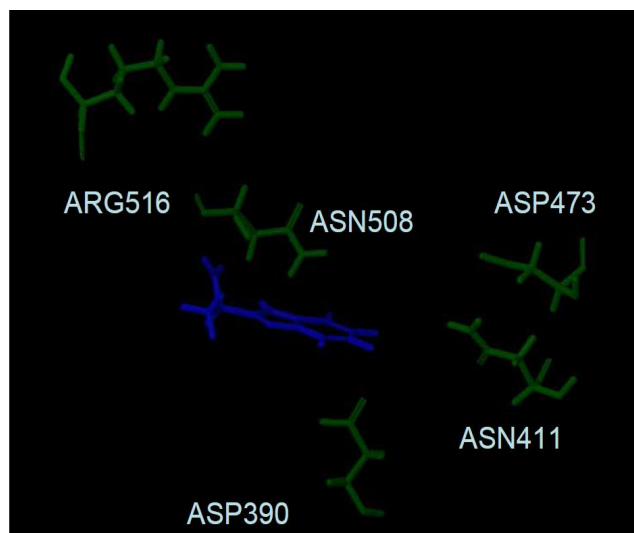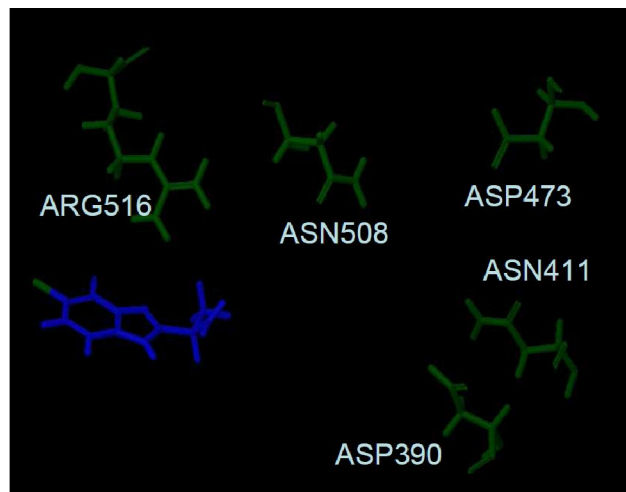

Figure 10 : Image of compound 5 docked in to the MTHF binding domain of MetS. (Front View)

Protein assay

The dose-dependent assay was performed to establish the amount of enzyme (protein) required to give a maximum of 20% conversion of the substrate into product. The plot from this experiment is shown in Figure 13. The assays were performed in duplicate and results are expressed as an average of the two individual values. Based on the results from these experiments an incubation time of 30 min and an enzyme dose of 0.74 mg were selected.

Enzyme assay

A time-dependent assay was used to identify the time range in which the linear reaction occurred using 0.74 mg of protein per assay. The plot from this experiment is shown by Figure 12. The assays were performed in duplicate and results are expressed as individual values. The results show that the parameters investigated are within the linear reaction.

Figure 11 : Image of compound 8 docked in to the MTHF binding domain of MetS. (Front View)

Determination of Km and Vmax for 5-methyltetrahydrofolate

Inhibition was studied with respect to varying 5-methyltetrahydrofolate concentrations. Assay incubation times had to be kept below 25 mins to ensure that the complete set of experimental data were collected within a 20% maximum of substrate conversion. The Lineweaver-Burk plot is shown in Figure 3. The value for K_M was calculated to be 28.6 μM and is consistent with the reported value of 25.0 μM.[7] Results are of three independent experiments, each one carried out in

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duplicate.

RESULTS AND DISCUSSION

The design of the synthesized molecules in this article was based on the structural similarity between the synthesized compounds and the natural substrate methyl tetrahydrofolate. The synthesized compounds compete with the natural substrate to its binding domain and this resulted in the inhibition of the enzyme reaction. A total number of 19 compounds of benzimidazole derivatives were synthesized in this article. Compound 6 showed the highest mixed *in vitro* inhibition of the free enzyme assay (K_i value of 450 µM and percentage inhibition 64%). The tested compounds were classified according to their degree of inhibition to the enzyme reaction. Compounds 6, 11 and 17 showed high inhibition action (64%, 41% and 40 %) respectively. Compounds 5 and 16 showed moderate inhibition action (37% and 36 % respectively). Compounds 3 and 14 showed the lowest inhibition action (3% and 22%). Compounds 1, 2, 4, 7, 8, 9, 10, 12, 13, 15 and 19 showed either stimulating or have no effect on the enzyme reaction (1 STM, 11 STM, 4 STM, 11 STM, 15 STM, 1 STM, 13 STM, 29 STM, 0, 0 and 11 STM) respectively.

Compound 6 showed a high inhibitory action on the enzyme catalyzed reaction (64%) and a low free energy of binding (-122.62 KJ/mol). Ligand 6 was docked with similar orientation as the docked pose of the natural substrate MTHF inside its binding domain with RMSD value of 1.60Å as illustrated in Figures 2 and 9. Where the nitro-benzenoid moiety of the ligand docked deep inside the receptor and the side chain at position 2 docked at the outermost part of the receptor. Compound 6 made 2 hydrogen bonds with the amino acids asparagine 508 and aspartic acid 390 (Figure 9). The low free energy of binding produced from the thermodynamic algorithms of the docked ligand resulted from the most plausible electrostatic and van der Waals interactions of the ligand with the key amino acids of the receptor. The steric hinderance at the minimum level and the polar side chain produced polar-nonpolar interactions with amino acid asparagine 508. Compound 5 exhibited a moderate inhibitory action on the enzyme catalyzed reaction (37%) and a moderate

free energy of binding (-81.93 KJ/mol). The absence of nitro group in ligand 5 had led to a remarkable reduction in the inhibition effect of the enzyme catalysed reaction and in the free energy of binding. This means that the presence of the nitro group at position 5 of benzimidazole ring is a detrimental bioisostere of amino group positioned at the pteridine ring of natural substrate MTHF. This led that the ligand started to rotate around its axis and docked horizontally inside the binding domain to avoid the boundary of steric clashes with other amino acids exist at the receptor cavity as shown in Figure 10. Ligand 8 unexpectedly stimulated the reaction catalysed with the enzyme (11% STM) and produced a high implausible free energy of binding (+29.37 KJ/mol). The presence of chloride atom as a bioisostere for the nitro group induced an undesired stimulating action of the enzyme activity and probably led to the stacking of the ligand at the surface of the enzyme cavity (Figure 11).
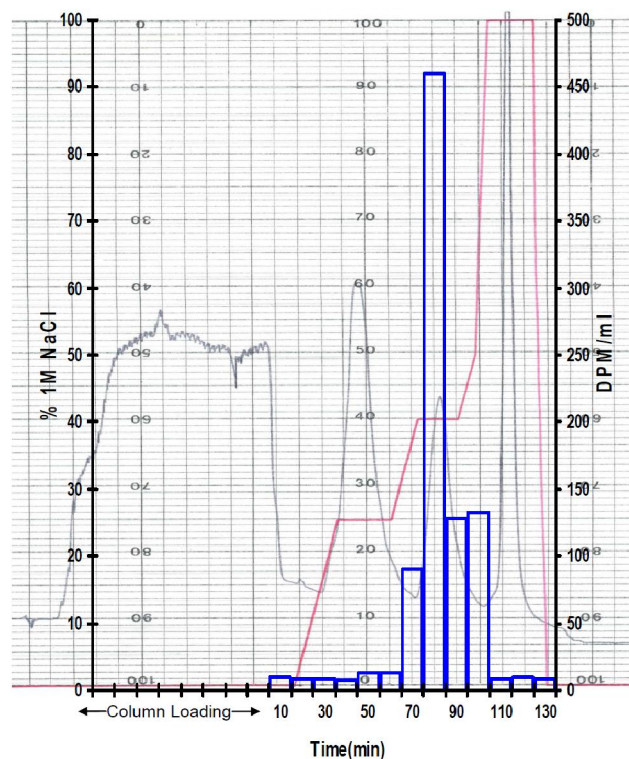

Figure 12 : Representative profile from Q-sepharose showing methionine synthase active fractions

A common route to the synthesis of benzimidazole derivatives is the well established Phillips condensation, reported by Phillips in 1928. The synthesis of compounds 1-25 followed alterations of known procedures. Substituted phenylenediamine derivatives were cyclized

Organic CHEMISTRY
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with different aliphatic and aromatic carboxylic acids to give the desired substituted benzimidazole derivatives. The reactions were enhanced in the presence of mineral acid catalyst.

Racemisation which possibly occurred during the synthesis of compounds 5-11 was examined by using a chiral shift reagent such as europium tris[3-(heptafluoro-propihydroxymethylene)-(+)-camphorate] . This reagent is able to interact with the stereogenic centre formed in compounds 5-11 to make physical complexes, converting the enantiomeric mixture if present into a mixture of diasteriomers which are different in the physical characters and could be detected using flash chromatography. This process has showed that one enantiomer was obtained and racemisation has not occurred.
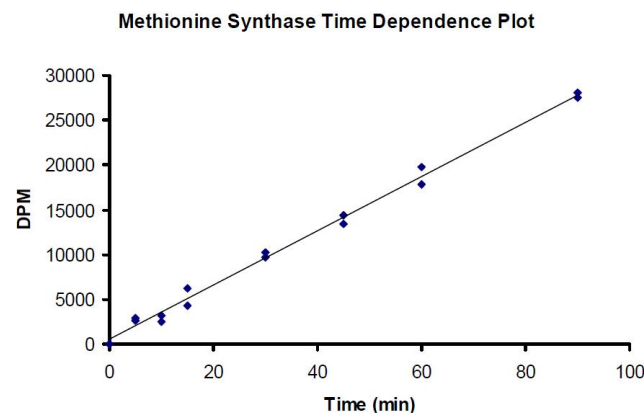
Figure 13 : Time-dependent study of methionine synthase (0.74 mg methionine synthase/ assay)

The target compounds that were successfully synthesized were tested for their inhibitory activity against highly purified rat liver cobalamin-dependent methionine synthase. A pilot study was undertaken on the synthesized series of substituted benzimidazole. All the compounds were tested using an established *in vitro* colourimetric enzyme assay[9]. The compounds were initially screened at 500 μM then at gradient concentrations of 500 μM, 250 μM and 100 μM. The kinetic parameters of the enzyme inhibition were calculated and Lineweaver-Burk plot for the uninhibited enzyme reactions for MTHF (K_M= 28.6 μM, V_{max}= 8.7 μM min^{-1}) was established (Figure 3). The tested compounds exhibited typical mixed inhibition patterns on lineweaver-burk plots (Figures 4-8). These results suggest that the inhibitors may bind at other binding sites as well as at the desired 5-methyltetrahydrofolate site. Methionine

synthase is a bisubstrate enzyme, it is possible that the inhibitors also compete for the homocysteine binding domain in addition to the 5-methyltetrahydrofolate binding domain, or even bind at the binding site of the *S*-adenosylmethionine reactivation cofactor. In this article we have only attempted the evaluation of the inhibitory activity against the 5-methyltetrahydrofolate binding site keeping all other parameters constant. Since there are structural similarities between the inhibitors and the side chain of the cobalamin cofactor it could be hypothesised that the inhibitors may displace the cofactor. In unbound cobalamin, the lower axial ligand is 5,6-dimethylbenzimidazole (Figure 14). When methionine docks its cobalamin cofactor, the 5,6-dimethylbenzi-midazole ligand is replaced with a histidine residue located on the active site. At every turnover of the enzyme, the histidine residue is removed then re-coordinated to the central cobalt atom. In theory, if the benzimidazole inhibitors or indeed any compound that had the ability to complex with cobalt gained access to the cobalamin binding domain, coordination could be achieved with the central cobalt in preference to the histidine residue, thereby blocking or hindering turnover. A reported hypothesis on the cleavage of the methyl group of cobalamin based on the strength of the axial ligand has been discussed in published article[10]. If this proposal stands, then it is reasonable to believe that a benzimidazole or a complexing ligand with a lower pK_a than the enzyme's histidine residue would decrease the strength of the ligand cobalt bond. In turn the cobalt methyl bond would be broken homolytically in favour to the heterolytic cleavage favoured and utilized by the enzyme. This could partially explain the inhibition seen by the benzimidazole species and the mixed behaviour of the kinetic studies. From a previous study, benzimidazole species with an electron-donating group on the benzene side typically increased the activity of the enzyme. Due to the electron-donating effect, the imidazole nitrogen atoms of these species would be more effective bases, hence would strengthen the ligand cobalt bond. If the theory holds, a strengthened ligand cobalt bond would favour heterolytic cleavage of the *trans* cobalt carbon bond. This outcome is preferred by the enzyme and so turnover could continue at a normal rate or be increased. This might simply be due to the fact that none of these compounds have a high affinity for the cobalamin bind-

ing site whilst benzimidazoles have been shown to complex with cobalt, as has 4-methoxy-1,2-phenylenediamine[11,12].

TABLE 1 : The calculated energy terms for the interaction of a series of compounds with MTHF binding domain and their percentage inhibition and their RMSD values

Ligand	Energy term (KJ/mol)				% Inhibition*	RMSD (Å)
	ΔEBind	ΔEComplex	ΔEReceptor	ΔELigand		
MTHF	-122.36	-255.00	-190.64	58.00	-	1.51
1	12.49	-202.72	-286.09	70.88	1STM	5.23
2	19.07	-227.08	-290.24	44.09	11STM	3.51
3	-2.91	-245.87	-285.05	42.09	3	3.12
4	5.09	-260.85	-298.42	32.48	4STM	4.67
5	-81.93	-301.15	-295.62	67.40	37	2.42
6	-122.62	-370.03	-326.11	78.70	64	1.60
7	28.95	-217.08	-293.04	47.01	11STM	4.90
8	29.37	-224.08	-294.20	40.79	15STM	6.81
9	13.01	-242.60	-316.59	60.98	1stm	6.20
10	1.87	-247.18	-299.24	50.19	13stm	2.51
11	-112.91	-275.87	-205.05	42.09	41	3.12
12	63.17	-204.88	-313.04	44.99	29STM	4.67
13	-0.34	-240.80	-284.15	43.69	0	4.42
14	13.91	-250.01	-299.21	35.29	22	1.6
15	7.70	-212.71	-289.49	69.08	0	1.9
16	-105.24	-272.00	-209.95	43.19	36	2.81
17	-114.80	-210.01	-176.09	80.88	40	5.23
18	-124.13	-260.08	-190.04	54.09	44	2.51
19	6.20	-245.03	-301.24	50.01	11STM	3.01
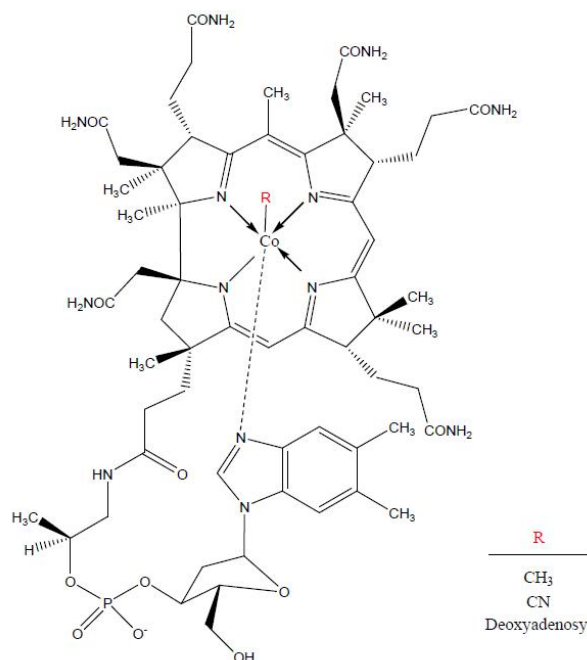
Figure 14 : Structure of cobalamin cofactor

TABLE 2 : Structure of MTHF and the compounds synthesized in this article

The structure of the compounds

Methyltetrahydrofolate (MTHF)

1

2

3

4
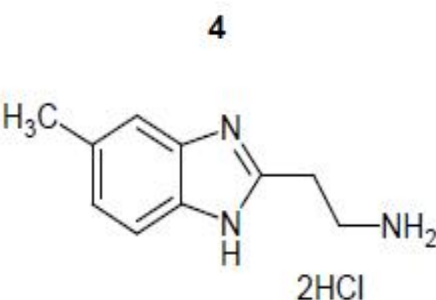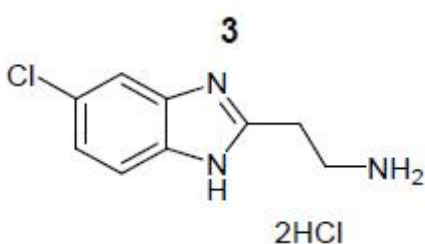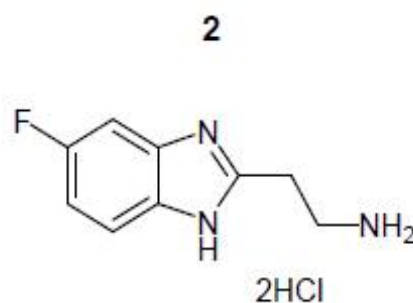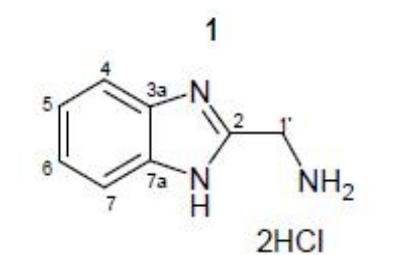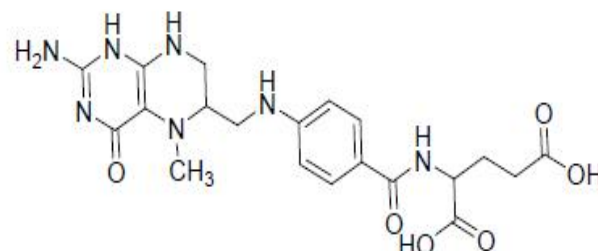
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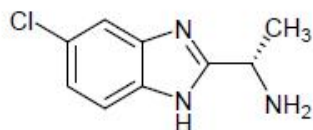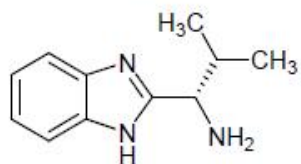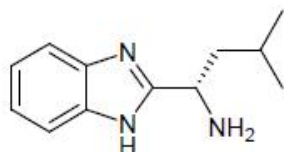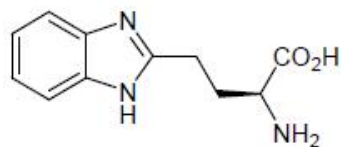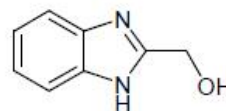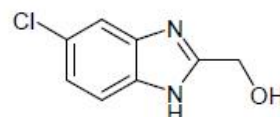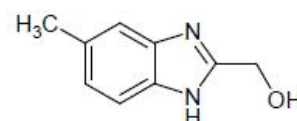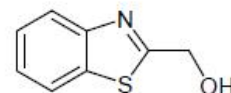| The structure of the compounds	The structure of the compounds
Methyltetrahydrofolate (MTHF)	Methyltetrahydrofolate (MTHF)
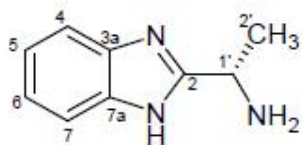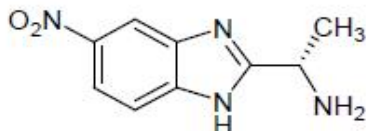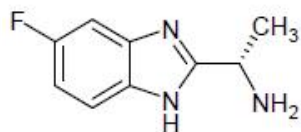
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2HCl

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2HCl

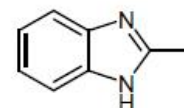**12**
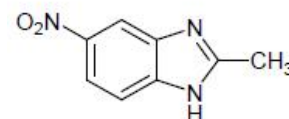

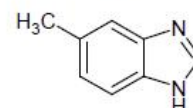
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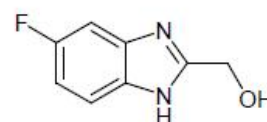
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Eq 1 The free energy of binding

$$\Delta E_{Bind} = \Delta E_{Complex} - (\Delta E_{Re\,ceptor} + \Delta E_{Ligand})\ (1)$$

Eq 2 Lineweaver-Burk equation for a non-competitive inhibitor

$$\frac{1}{V_0} = \frac{K_M(1+[I]/K_i)}{V_{max}}\frac{1}{[S]} + \frac{K_i'}{V_{max}} \qquad (2)$$

Eq 3 Lineweaver-Burk equation for an un-competitive inhibitor

$$\frac{1}{V_0} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1+[I]/K_i'}{V_{max}} \qquad (3)$$

CONCLUSION

The results obtained from this study showed new generation of compounds that could probably used as drugs in the treatment of breast and prostate carcinoma. The 5-methyltetrahydrofolate (MTHF) binding domain is considered a big receptor (20.70Å length and 12.23Å width) and has five key amino acid residues which take part in the binding, and comprise of negatively charged amino acids aspartic acid 390 and 473, polar uncharged amino acids asparagine 411 and 508 and positively charged amino acid arginine 516. The five key amino acid residues comprising the binding site of MTHF are polar, three of them are charged and two are uncharged. This means that any design of inhibitor mimics should take into account the presence of polar and charged groups or atoms in the inhibitors in order to facilitate the formation of strong Coloumb, Lennard-Jones 6-12 potentials and formation of hydrogen bonds.

The MTHF binding pocket showed a very interesting distribution of amino acid residues. The charged and polar amino acids were populated in the outermost part of the pocket and the nonpolar hydrophobic amino acids are located in the middle part of the pocket. Therefore the benzimidazole derivatives designed in this article, and for the future, work should take into account the presence of a nitro group as a substituent on the benzenoid ring of the inhibitor. The nitro group occupied the cartesian coordinates, where the charged and polar amino acids are populated. This enhanced the ligand-receptor binding via charged-charged electrostatic interactions. Some benzimidazole derivatives showed a meaningful inhibition of the methionine synthase reaction. The synthesized ligands exhibited different inhibitory activity on the enzyme reaction with low concentrations. Testing against particular cancer cells where methionine synthase is over-expressed will be required for the subsequent step.

ACKNOWLEDGMENT

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