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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Molecular modeling studies and synthesis of novel quinoxaline derivatives with potential anti-cancer activity as inhibitors of methionine synthase

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**Abstract** Methionine synthase (MetS) catalyses the transfer of a methyl group from the methyltetrahydrofolate (MTHF) to homocysteine to produce methionine and tetrahydrofolate. MetS is over-expressed in the cytosol of certain breast and prostate tumor cells. In this article, we designed, synthesized, and evaluated the biological activity of a series of substituted quinoxaline derivatives that mimic the MTHF in the structure. The main aim was to develop inhibitors that zcould inhibit the enzyme reaction by blocking the binding of MTHF. These inhibitors were docked into the MTHF binding domain in such the same way as MTHF in its binding domain. Compound 4-({(6-nitro-quinoxalin-2-yl)methylamino}methyl)benzoic acid showed the lowest free energy of the binding (−152.62 kJ/mol) and showed the lowest $IC_{50}$ values of 45 ± 9 and 53 ± 9 μM against two types of cancer cell lines PC-3 and MCF-7, respectively.

**Keywords** Cobalamin · MCF-7 cells · Methionine · Methionine synthase · PC-3 · Quinoxaline derivatives and tumor

## 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 (Halpern *et al.*, 1974). 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 (Fig. 1). Crystal structure of cobalamin-dependent MetS revealed that it consists of four functional binding domains. They are homocysteine binding domain, 5-methyltetrahydrofolate binding domain, the third domain binds cobalamin cofactor, and the fourth domain is an allosteric cofactor *S*-adenosy-L-methionine (S-AdoMet) (Hoffman, 1982). The reaction products methionine and tetrahydrofolate (THF) are closely correlated to important biochemical reactions of the methylation of DNA, lipids, proteins, and polyamine (Kenyon *et al.*, 2002; Pavillard *et al.*, 2006). Some recent studies have showed promising anti-tumor and apoptosis activities in colon with *S*-adenosylmethionine (SAMe) and its metabolite methylthioadenosine (MTA) (Tony *et al.*, 2011). Some investigations using quinoxaline derivatives such as 3-aryl-2-quinoxaline-carbonitrile 1,4-di-*N*-oxide derivatives showed hypoxic and cytotoxic activities against some of human cell lines (Yunzhen *et al.*, 2012).

The present study showed a new approach for determining specific inhibitors of MetS. Quinoxaline derivatives that resemble substructure of 5-MTHF (Fig. 2) have been docked into 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 and in vitro tumor cell cytotoxicity assay.

## Materials and methods

### Molecular modeling procedure

Molecular modeling was carried out on Schrodinger computational software workstation using Maestro 7.5 graphic

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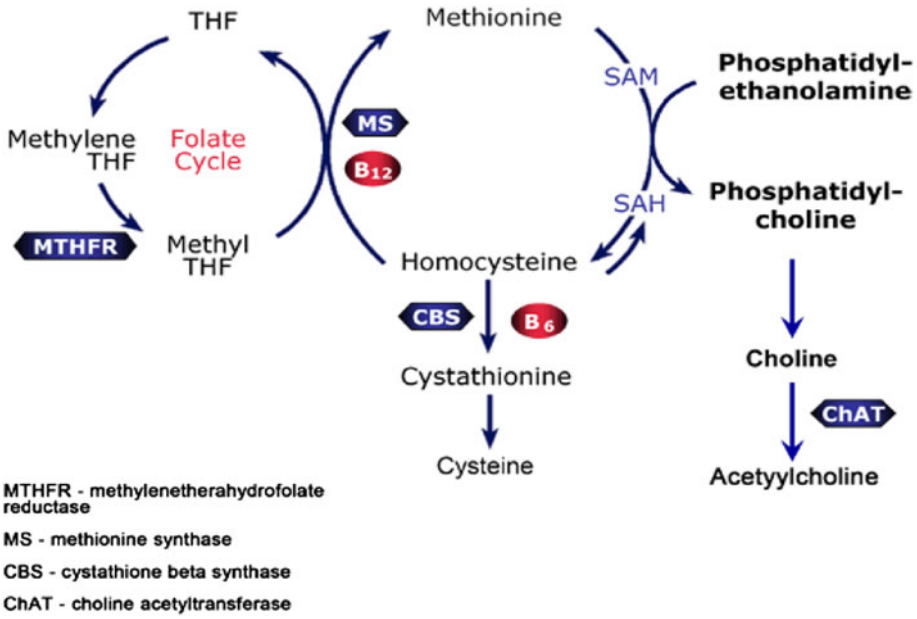



**Fig. 1** Different enzymes involved in the folic acid cycle. The *cycle* starts with THF and ends up with methyltetrahydrofolate (MTHF) which is in turn used by methionine synthase as a substrate and metabolizes it to produce essential amino acid methionine

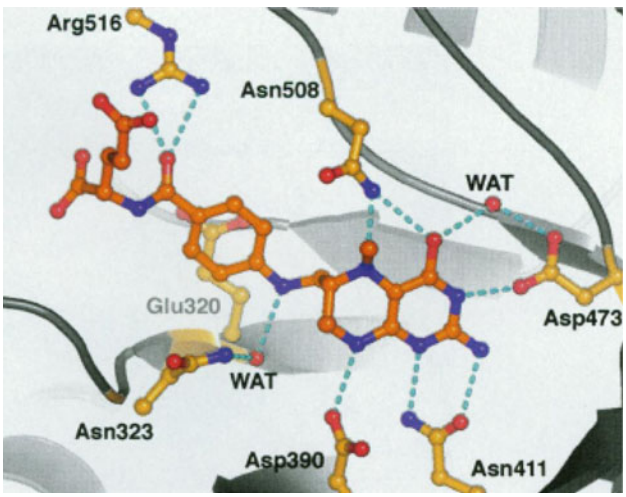



**Fig. 2** The interception of MTHF with its binding domain of methionine synthase forming hydrogen bonding with the key amino acids of the receptor. The *image* showed that MTHF is docked horizontally deep inside the receptor where the pteridine nucleus is positioned at *right hand side* and the aromatic and γ glutamate side chain at the orifice of the receptor at *left hand side*

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 4 Å distance from the homocysteine binding site. The basic and acidic amino acids were neutralized at pH 7 ± 2 by protonation of the terminal amino groups and basic amino acids 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 anticipated to have poor energies would be rejected from the conditioned set up of the hierarchal filter. SP docking is appropriate for screening ligands of unknown quality in large numbers. SP is a soft docking program 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 penalized. 5,000 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 fulfill those conditions were subjected to energy minimization on the OPLS-AA non-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 (Cairns *et al.*, 2001).

## Experimental procedure

4-Nitro-phenylen-1,2-diamine and 2-iodobenzoic acid (IBX) were obtained from Aldrich (Poole, UK). Aromatic amine compounds were obtained from Avocado (Hewsham, UK). 3-Chloro-2-oxopropanal was gifted from a colleague. Deuterated solvents were from Goss (Glossop, UK). Melting points were determined using an Electrothermal IA9200 digital melting point apparatus. IR spectra were recorded on a Perkin Elmer (Paragon 1000) FT-IR Spectrophotometer (Perkin Elmer, Seer Green, UK). $^{1}H$ and $^{13}C$ NMR spectra were acquired at 270.05 and 67.80 MHz, respectively, on a JEOL GX270 spectrometer (JEOL UK, Welwyn, UK); 13C assignments were made using the DEPT135 experiment, and coupling constant (J) was measured in hertz. Mass spectra were obtained from IPI, University of Bradford, West Yorkshire, UK.

### *2-Chloromethyl-6-nitroquinoxaline [1]*

A solution of 4-nitro-phenylen-1,2-diamine (0.223 g, 1 mmol) and 3-chloro-2-oxopropanal (0.104 g, 1 mmol) in dry glacial acetic acid (5 mL) was stirred at rt in the with catalytic amount of IBX (0.028 g, 0.01 mmol). The reaction mixture was filtered. $NaHCO_3$ (5 mL, 5 % g/v) was added to the filtrate and extracted with diethyl ether (2 × 5 mL). The combined organic layers were washed with brine (2 × 5 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the crude product was crystallized with ethanol to give the product as bright yellow crystals (0.13 g, 58 %); m.p. 213–216 °C (lit. 215–216 °C) (Majid and Khadijeh, 2006).

$\delta_H$ (DMSO-$d_6$): 8.48 (1 H, d, $J^4$ 1.2, H-6), 8.16 (1 H, s, H-3), 7.75 (1H, dd, $J^3$ 1.2, $J^4$ 2.3, H-8), 7.58 (1 H, d, $J^3$ 2,3, H-9), 4.62 (2 H, s, $CH_2$), NH signal was not observed; $\delta_C$ (DMSO-$d_6$): 158 (C-2), 148 (C-7), 147 (C-3), 144 (C-10), 140 (C-5), 130 (C-9), 125 (C-6), 122 (C-8), 45 ($CH_2$); DEPT 135 (DMSO-$d_6$): 147 (C-3), 130 (C-9), 125 (C-6), 122 (C-8), 45 ($CH_2$); $\upsilon_{max}/cm^{-1}$ (KBr): 3098 (C–H, $sp^2$), 2891 (C–H, $sp^3$), 1300 (C–H, bend); MS (ES): *m/z* 224 ($M^{+\bullet}$, 95 %), 131 (11), 119 (8); Elemental analysis: Found: C, 48.42; H, 2.73; N; 18.27. $C_9H_6ClN_3O_2$ requires: C, 48.34; H, 2.70; N, 18.79 %.

### *N-[(6-Nitroquinoxalin-2-yl)methyl]benzenamine [2]*

Aniline (2.21 mL, 0.238 mmol) dissolved in Tetrahydrofuran (25 mL) and triethylamine (2 mL) was added to the stirred solution. 2-Chloromethyl-6-nitroquinoxaline (3.33 g, 0.149 mmol) was added dropwise to the stirred solution, which was then heated under reflux for 100 h. The reaction mixture was evaporated under reduced pressure. The crude product was dissolved in dichloromethane and washed with water (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield a brown precipitate, which recrystallized from acetone to give the product as bright yellow crystals (2.12 g, 63.6 %); m.p. 235–237 °C (Woo-Jin and Chao-Jun, 2006).

$\delta_H$ (DMSO-$d_6$): 9.22 (1 H, s, H-6), 8.93 (1 H, s, H-3), 8.75 (1 H, d, $J^3$ 1.4, H-8), 8.01 (1 H, d, $J^3$ 1.4, H-9), 7.25–6.34 (5 H, m, H-14–18), 3.62 (2 H, s, H-11), NH signal was not observed; $\delta_C$ (DMSO-$d_6$): 160 (C-2), 152 (C-7), 150 (C-3), 148 (C-13), 147 (C-10), 146 (C-5), 137 (C-9), 136 (C-15, C-17), 130 (C-6), 128 (C-8), 120 (C-16), 118 (C-14, C-18) 35 (C-11); DEPT 135 (DMSO-$d_6$): 137 (C-9), 136 (C-15, C-17), 130 (C-6), 128 (C-8), 120 (C-16), 118 (C-14, C-18) 35 (C-11); $\upsilon_{max}/cm^{-1}$ (KBr): 3120 (N–H), 3122 (C–H, $sp^2$), 2722 (C–H, $sp^3$), 1378 (C–H, bend); MS (ES): *m/z* 281 ($M^{+\bullet}$, 100 %), 210 (30), 187 (40), 166 (30); Elemental analysis: Found: C, 64.44; H, 4.33; N; 19.72. $C_{15}H_{12}N_4O_2$ requires: C, 64.28; H, 4.32; N, 19.99 %.

### *4-Methyl-N-[(6-nitroquinoxalin-2-yl)methyl] benzenamine [3]*

Method 2.2 was followed using *P*-toluidine (0.25 g, 0.238 mmol) dissolved in absolute ethanol (25 mL), triethylamine (2 mL), and 2-chloromethyl-6-nitroquinoxaline (3.33 g, 0.149 mmol). The reaction mixture was heated under reflux for 79 h and then evaporated under reduced pressure. The crude product was dissolved in ethyl acetate and washed with water (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield a yellow precipitate, which recrystallized from ethanol to give the product as bright yellow crystals (0.14 g, 56 %); m.p. 270–272 °C (Woo-Jin and Chao-Jun, 2006).

$\delta_H$ (DMSO-$d_6$): 8.76–6.32 (8 H, m, H-3, H-6, H-8, H-9, H-14, H-15, H-17, H-18), 3.81 (2 H, s, H-11), NH signal was not observed; $\delta_C$ (DMSO-$d_6$): 158 (C-2), 156 (C-7), 152 (C-3), 144 (C-10, C-13), 138 (C-5), 137 (C-9), 129 (C-15, C-17), 126 (C-16), 126 (C-6), 123 (C-8), 116 (C-14,

C-18), 43 (C-11), 25 (C-19); DEPT 135 (DMSO-$d_6$): 152 (C-3), 129 (C-15, C-17), 137 (C-9), 126 (C-6), 123 (C-8), 116 (C-14, C-18) 43 (C-11), 25 (C-19); MS (ES): *m/z* 295 ($M^{+\bullet}$, 50 %), 230 (20), Elemental analysis: Found: C, 65.00; H, 4.34; N; 19.01. $C_{16}H_{14}N_4O_2$ requires: C, 65.30; H, 4.79; N, 19.04 %.

### *3-Methyl-N-[(6-nitroquinoxalin-2-yl)methyl] benzenamine [**4**]*

Method 2.2 was followed using *m*-toluidine (0.25 g, 0.238 mmol) dissolved in absolute ethanol (25 mL), triethylamine (2 mL), and 2-chloromethyl-6-nitroquinoxaline (3.33 g, 0.149 mmol). The reaction mixture was heated under reflux for 74 h and then concentrated under reduced pressure. The crude product was dissolved in ethyl acetate and washed with water (3 × 10 mL). The organic layer was evaporated to give a yellow precipitate, which recrystallized from ethanol to give the product as bright yellow crystals (0.06 g, 24 %); m.p. 266–267 °C (Woo-Jin and Chao-Jun, 2006).

$\delta_H$ (DMSO-$d_6$): 8.46–6.32 (8 H, m, H-3, H-6, H-8, H-9, H-14, H-15, H-17, H-18), 4.01 (2 H, s, H-11), NH signal was not observed; $\delta_C$ (DMSO-$d_6$): 161 (C-2), 156 (C-7), 155 (C-3), 147 (C-13), 143 (C-5, C-10), 140 (C-9), 139 (C-15), 134 (C-17), 130 (C-16), 124 (C-6), 123 (C-8), 117 (C-14), 116 (C-18), 40 (C-11), 23 (C-19); DEPT 135 (DMSO-$d_6$): 155 (C-3), 137 (C-9), 134 (C-17), 130 (C-16), 124 (C-6), 123 (C-8), 117 (C-14), 116 (C-18), 40 (C-11), 23 (C-19); MS (ES): *m/z* 295 ($M^{+\bullet}$, 60 %), 220 (33), 100 (20); Elemental analysis: Found: C, 65.40; H, 4.81; N; 19.05. $C_{16}H_{14}N_4O_2$ requires: C, 65.30; H, 4.79; N, 19.04 %.

### *4-Chloro-N-[(6-nitroquinoxalin-2-yl)methyl] benzenamine [**5**]*

Method 2.2 was followed using *p*-chloro-aniline (0.303 g, 0.238 mmol) dissolved in THF (30 mL), triethylamine (2 mL), and 2-chloromethyl-6-nitroquinoxaline (3.33 g, 0.149 mmol). The reaction mixture was heated under reflux for 100 h and then concentrated under reduced pressure. The crude product was dissolved in ethyl acetate and washed with water (3 × 10 mL). The organic layer was evaporated to give a brown precipitate, which recrystallized from acetone to give the product as bright yellow crystals (0.06 g, 19 %); m.p. 242–244 °C (Woo-Jin and Chao-Jun, 2006).

$\delta_H$ (DMSO-$d_6$): 9.01 (1 H, s, H-6), 8.95 (1 H, s, H-3), 8.75 (1 H, dd, $J^3$ 1.5, $J^4$ 2.5, H-8), 8.50 (1 H, d, $J^3$ 1.5, H-9), 8.00–6.30 (5 H, m, H-14, H-15, H-17, H-18), 4.02 (2 H, s, H-11), NH signal was not observed; $\delta_C$ (DMSO-$d_6$): 162 (C-2), 155 (C-7), 152 (C-3), 150 (C-13), 142 (C-10), 140 (C-5), 137 (C-9), 136 (C-15, C-17), 132 (C-6), 130 (C-8), 122 (C-16), 120 (C-14, C-18) 42 (C-11); DEPT 135 (DMSO-$d_6$): 152 (C-3), 137 (C-9), 136 (C-15, C-17), 132 (C-6), 130 (C-8), 122 (C-16), 120 (C-14, C-18) 42 (C-11); MS (ES): *m/z* 316 ($M^+$+1, 66 %), 300 (100), 122 (40), 166 (30); Elemental analysis: Found: C, 57.22; H, 3.90; N; 17.72. $C_{15}H_{11}ClN_4O_2$ requires: C, 57.24; H, 3.53; N, 17.80 %.

### *4-[(6-Nitroquinoxalin-2-yl)methylamino)benzoic acid [**6**]*

Method 2.2 was followed using p-amino-benzoic acid (0.137 g, 0.01 mmol) dissolved in THF (25 mL), triethylamine (2 mL), and 2-chloromethyl-6-nitroquinoxaline (0.223 g, 0.01 mmol). The reaction mixture was heated under reflux for 200 h and then concentrated under reduced pressure. The crude product was dissolved in dichloromethane and washed with water (3 × 10 mL). The organic layer was evaporated to give a brown precipitate, which recrystallized from methanol to give the product as brown crystals (0.2 g, 40 %); m.p. 215–216 °C (Woo-Jin and Chao-Jun, 2006).

$\delta_H$ (DMSO-$d_6$): 9.51 (1 H, brs, COOH), 9.01 (1 H, s, H-6), 8.63 (1 H, s, H-3), 8.75 (1 H, d, $J^3$ 1.4, H-8), 8.12 (1 H, d, $J^3$ 1.6, H-9), 7.54–6.38 (4 H, m, H-14, H-15, H-17, H-18), 4.62 (2 H, s, H-11), 2.1 (1 H, brs, H-12); $\delta_C$ (DMSO-$d_6$): 168 (C-19), 162 (C-2), 153 (C-13), 151 (C-7), 148 (C-3), 145 (C-10), 143 (C-5), 140 (C-15, C-17), 138 (C-9), 135 (C-6), 131 (C-8), 128 (C-16), 121 (C-14, C-18), 34 (C-11); DEPT 135 (DMSO-$d_6$): 148 (C-3), 140 (C-15, C-17), 138 (C-9), 135 (C-6), 131 (C-8), 121 (C-14, C-18), 34 (C-11); $\upsilon_{max}/cm^{-1}$ (KBr): 3510 (O–H), 3155 (N–H), 3210 (C–H, $sp^2$), 2756 (C–H, $sp^3$), 1421 (C–H, bend); MS (ES): *m/z* 325 ($M^{+\bullet}$, 66 %), 300 (45), 210 (40), 150 (30); Elemental analysis: Found: C, 59.00; H, 3.33; N; 17.12. $C_{16}H_{12}N_4O_4$ requires: C, 59.26; H, 3.73; N, 17.28 %.

### *4-[(6-Nitroquinoxalin-2-yl)methylamino)benzoyl chloride [**7**]*

4-[(6-Nitroquinoxalin-2-yl)methylamino)benzoic acid (0.402 g, 1.24 mmol) was dissolved in dry dichloromethane (25 mL). Oxalyl chloride (0.15 mL, 1.24 mmol) was added dropwise to the stirred solution and the mixture was left stirring for 6 h. The solvent was removed under reduced pressure to afford a light yellow precipitate, which was recrystallized from dry dichloromethane to give a product with white powder (0.23 g, 76 %); m.p. 90–92 °C (Kitamura and Yoshida, 2003).

$\delta_H$ (DMSO-$d_6$): 8.85 (1 H, s, H-6), 8.60 (1 H, s, H-3), 8.77 (1 H, d, $J^3$ 1.4, H-8), 8.12 (1 H, d, $J^3$ 1.6, H-9), 7.53–6.66 (4 H, m, H-14, H-15, H-17, H-18), 4.62 (2 H, s, H-11), 1.7 (1 H, brs, H-12).

**Table 1** The calculated energy terms for the interaction of a series of compounds with MTHF binding domain and their RMSD values

| Ligand | Energy term (kJ/mol) | | | | $IC_{50}$ (μM) | | RMSD (Å) |
|---|---|---|---|---|---|---|---|
| | $\Delta E_{Bind}$ | $\Delta E_{Complex}$ | $\Delta E_{Receptor}$ | $\Delta E_{Ligand}$ | PC-3 | MCF-7 | |
| **1** | −105.51 | −202.72 | −186.09 | 88.88 | 70 ± 19 | 66 ± 19 | 5.23 |
| **2** | −130.93 | −267.08 | −190.24 | 54.09 | 56 ± 8 | 61 ± 6 | 2.51 |
| **3** | −132.91 | −275.87 | −185.05 | 42.09 | 57 ± 14 | 68 ± 14 | 3.12 |
| **4** | −138.91 | −290.85 | −218.42 | 66.48 | 77 ± 10 | 79 ± 14 | 4.67 |
| **5** | −122.93 | −341.15 | −285.62 | 67.40 | 70 ± 13 | 75 ± 4 | 4.42 |
| **6** | −152.62 | −390.03 | −326.11 | 88.70 | 45 ± 9 | 53 ± 9 | 1.6 |
| **8** | −150.19 | −331.05 | −280.94 | 100.08 | 49 ± 6 | 47 ± 6 | 1.9 |
| **9** | −145.89 | −341.55 | −299.94 | 104.28 | 66 ± 6 | 49 ± 9 | 2.81 |

The table represents a collection of information about the binding algorithms of the different docked ligands into the receptor

$\Delta E_{Bind}$ The free binding energy algorithm results from Eq. 1

$\Delta E_{Complex}$ The free binding energy of the ligand-receptor complex

$\Delta E_{Receptor}$ The free binding energy of the receptor

$\Delta E_{Ligand}$ The free binding energy of the ligand

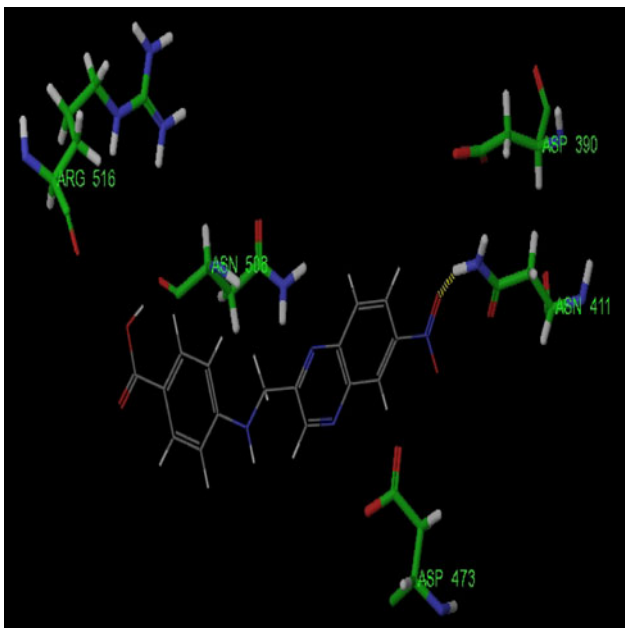



**Fig. 3** Image of compound 6 docked into the MTHF binding domain of MetS. The *left hand side* represents the outermost part of the enzyme and the orifice of the receptor and the *right hand side* represents the innermost part of the enzyme. Compound 6 makes hydrogen bond with asparagine 411

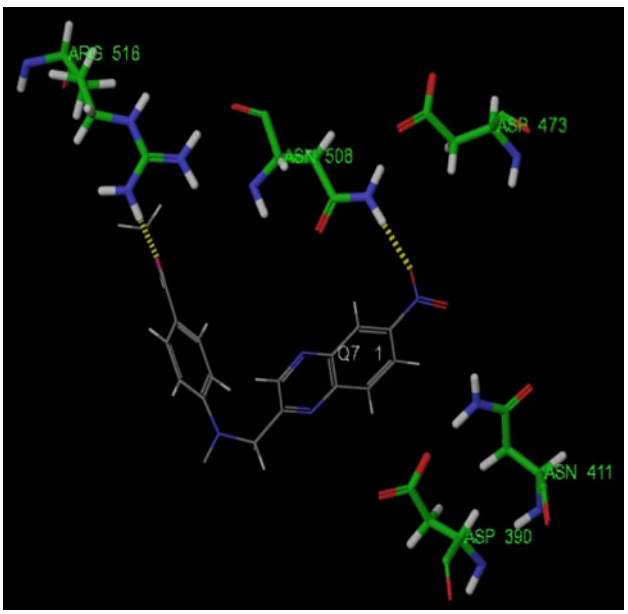



**Fig. 4** Image of compound 8 docked into the MTHF binding domain of MetS. The *left hand side* represents the outermost part of the enzyme and the orifice of the receptor and the *right hand side* represents the innermost part of the enzyme. Compound 8 makes hydrogen bonds with arginine 516 and asparagine 508

*4-[(6-Nitroquinoxalin-2-yl)methylamino)-N-methylbenzamide [8]*

Method 2.2 was followed using methylamine (0.031 mL, 0.01 mmol) dissolved in THF (25 mL), triethylamine (2 mL), and 4-[(6-nitroquinoxalin-2-yl)methylamino)benzoyl chloride (0.342 g, 0.01 mmol). The reaction mixture was heated under reflux for 12 h and concentrated under reduced pressure. The crude product was dissolved in dichloromethane and washed with water (3 × 10 mL). The organic layer was evaporated to give a brown precipitate, which recrystallized from methanol to give the product as brown crystals (0.11 g, 32 %); m.p. 260–261 °C (Woo-Jin and Chao-Jun, 2006).

$\delta_H$ (DMSO-$d_6$): 9.06 (1 H, s, H-6), 8.68 (1 H, s, H-3), 8.60 (1 H, dd, $J^3$ 2.6, $J^4$ 1.2, H-8), 8.07 (1 H, d, $J^3$ 2.6, H-9), 8.00 (1 H, br s, H-20), 7.34–6.78 (4 H, m, H-14, H-15,

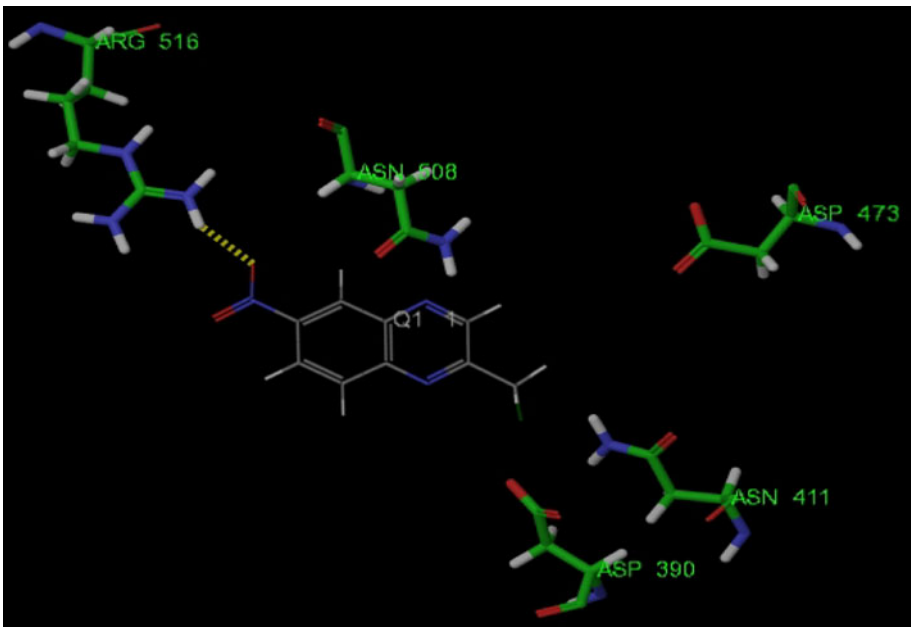



**Fig. 5** Image of compound 1 docked into the MTHF binding domain of MetS. The *left hand side* represents the outermost part of the enzyme and the orifice of the receptor, and the *right hand side* represents the innermost part of the enzyme. Compound 1 makes hydrogen bond with arginine 516

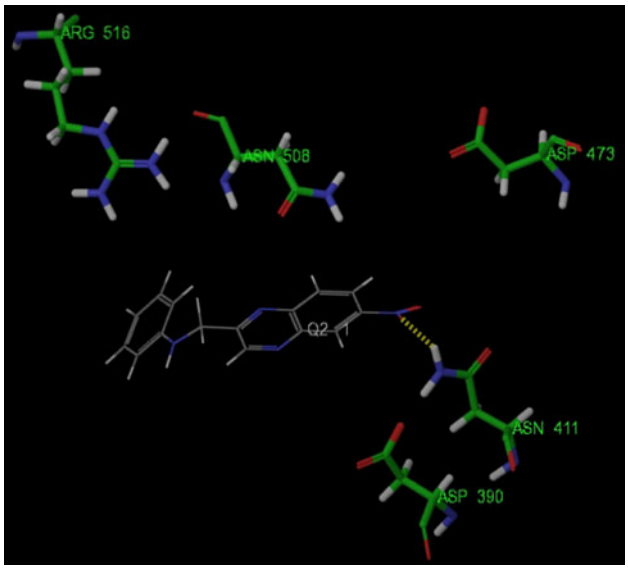



**Fig. 6** Image of compound 2 docked into the MTHF binding domain of MetS. The *left hand side* represents the outermost part of the enzyme and the orifice of the receptor, and the *right hand side* represents the innermost part of the enzyme. Compound 2 makes hydrogen bond with asparagine 411

H-17, H-18), 4.32 (2 H, s, H-11), 4.1 (1 H, br s, H-12), 2.9 (3 H, s, H-21); $\delta_C$ (DMSO-$d_6$): 169 (C-19), 164 (C-2), 155 (C-13), 153 (C-7), 150 (C-3), 146 (C-10, C-5), 133 (C-15, C-17), 132 (C-9), 131 (C-6), 130 (C-8), 128 (C-16), 127 (C-14, C-18), 35 (C-11), 26 (C-21); DEPT 135 (DMSO-$d_6$): 150 (C-3), 133 (C-15, C-17), 132 (C-9), 131 (C-6), 130 (C-8), 127 (C-14, C-18), 34 (C-11), 26 (C-21); MS (ES): *m/z* 338 ($M^{+\bullet}$, 71 %), 305 (40), 200 (30), 150 (30); Elemental analysis: Found: C, 60.02; H, 4.33; N; 20.52. $C_{17}H_{15}N_5O_3$ requires: C, 60.53; H, 4.48; N, 20.76 %.

### *4-[(6-Nitroquinoxalin-2-yl)methylamino)-N-ethylbenzamide* ***[9]***

Method 2.2 was followed using ethylamine (0.045 mL, 0.01 mmol) dissolved in THF (25 mL), triethylamine (2 mL), and 4-[(6-nitroquinoxalin-2-yl)methylamino)benzoyl chloride (0.342 g, 1 mmol). The reaction mixture was heated under reflux for 22 h and concentrated under reduced pressure. The crude product was dissolved in dichloromethane and washed with water (3 × 10 mL). The organic layer was evaporated to give a brown precipitate, which recrystallized from methanol to give the product as brown crystals (0.10 g, 29 %); m.p. 255–256 °C (Woo-Jin and Chao-Jun, 2006).

$\delta_H$ (DMSO-$d_6$): 8.66 (1 H, s, H-6), 8.63 (1 H, s, H-3), 8.60 (1 H, d, $J^3$ 2.9, H-8), 8.12 (1 H, d, $J^3$ 2.9, H-9), 7.57–6.38 (4 H, m, H-14, H-15, H-17, H-18), 4.62 (2 H, s, H-11), 2.2 (1 H, br s, OH), 2.1 (1 H, brs, H-12), 3.2 (2 H, q, $J^3$ 3.2, H-21), 3.00 (1 H, br s, H-20), 1.2 (3 H, t, $J^3$ 3.2, H-22); $\delta_C$ (DMSO-$d_6$): 170 (C-19), 163 (C-2), 160 (C-13), 158 (C-7), 153 (C-3), 145 (C-10, C-5), 145 (C-15, C-17), 144 (C-9), 130 (C-6), 129 (C-8), 128 (C-16), 127 (C-14, C-18), 37 (C-11), 26 (C-21), 20 (C-22); DEPT 135 (DMSO-$d_6$): 153 (C-3), 145 (C-15, C-17), 144 (C-9), 130 (C-6), 129 (C-8), 127 (C-14, C-18), 37 (C-11), 26 (C-21), 20 (C-22); MS (ES): *m/z* 352 ($M^{+\bullet}$, 90 %), 320 (50), 200 (30), 150 (30); Elemental analysis: Found: C, 61.34; H, 4.45; N; 20.02. $C_{18}H_{17}N_5O_3$ requires: C, 61.53; H, 4.88; N, 19.93 %.

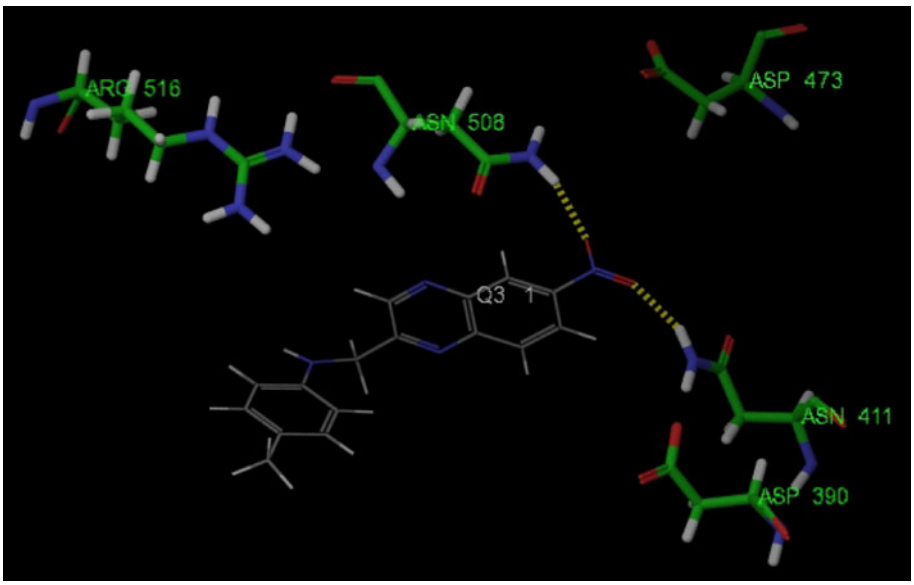



**Fig. 7** Image of compound 3 docked into the MTHF binding domain of MetS. The *left hand side* represents the outermost part of the enzyme and the orifice of the receptor, and the *right hand side* represents the innermost part of the enzyme. Compound 3 makes hydrogen bonds with asparagine 411 and asparagine 508

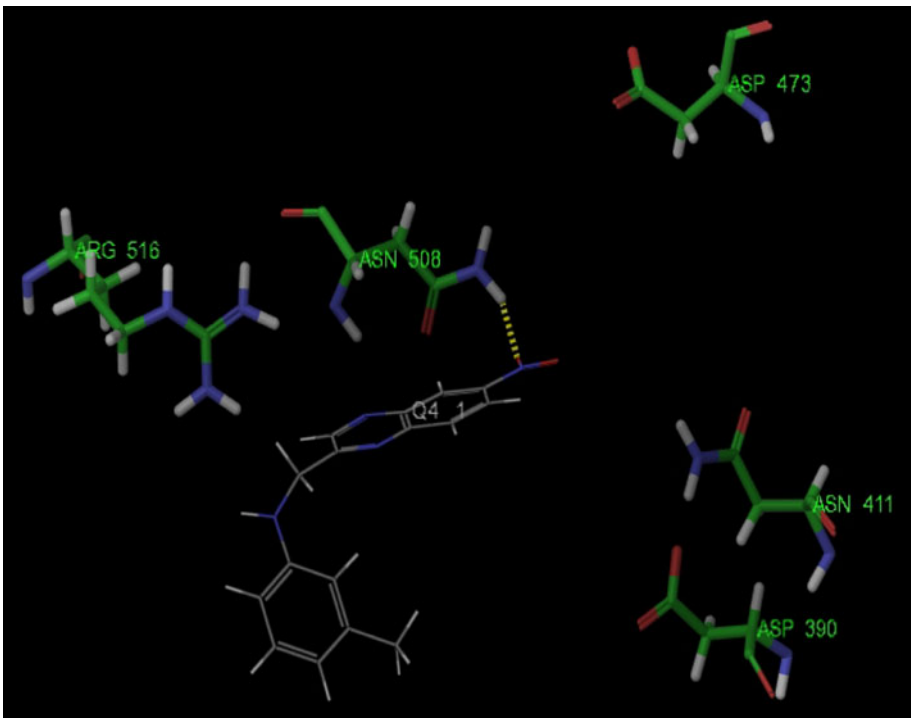



**Fig. 8** Image of compound 4 docked into the MTHF binding domain of MetS. The *left hand side* represents the outermost part of the enzyme and the orifice of the receptor, and the *right hand side* represents the innermost part of the enzyme. Compound 4 makes hydrogen bond with asparagine 508

## Biological evaluation

Cell lines were supplied by the European Collection of Cell Cultures (ECACC); human prostate adenocarcinoma cells (PC-3) and breast carcinoma (MCF-7) were investigated. Cells were preserved in vitro at 37 °C in a 5 % carbon dioxide, 95 % air dampened incubator. Cells were grown in RPMI media containing 100 μM of *l*-methionine (abbreviated M + H) or methionine-free RPMI media containing 100 μM of *l*-homocysteine thiolactone (abbreviated $M–H^+$). Media was supplemented with 10 % 1 kDa cut-off dialyzed fetal calf serum (HL60, 20 %), sodium pyruvate (1 mM), L-glutamine (2 μM), hepes (24 μM), and sodium bicarbonate (2 μM). Cells in exponential growth were plated (500–1,000 $\times$ $10^{-3}$ cells per well) in complete $M + H^-$, medium, containing L-methionine (100 μM). After an initial incubation of 24 h at 37 °C, the medium was changed to $M–H^-$ and cells were treated with

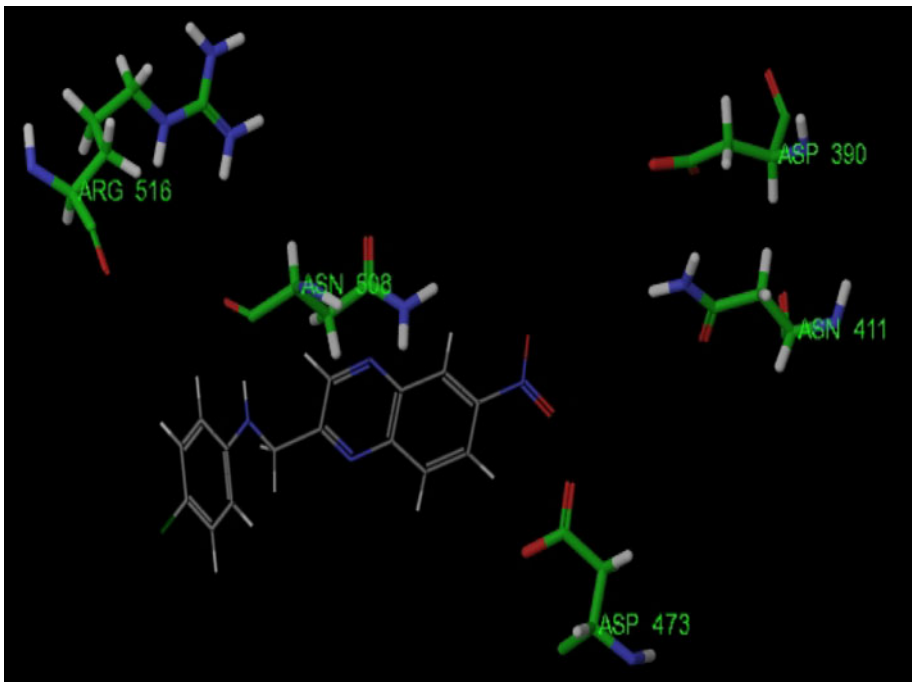



**Fig. 9** Image of compound 5 docked into the MTHF binding domain of MetS. The *left hand side* represents the outermost part of the enzyme and the orifice of the receptor, and the *right hand side* represents the innermost part of the enzyme

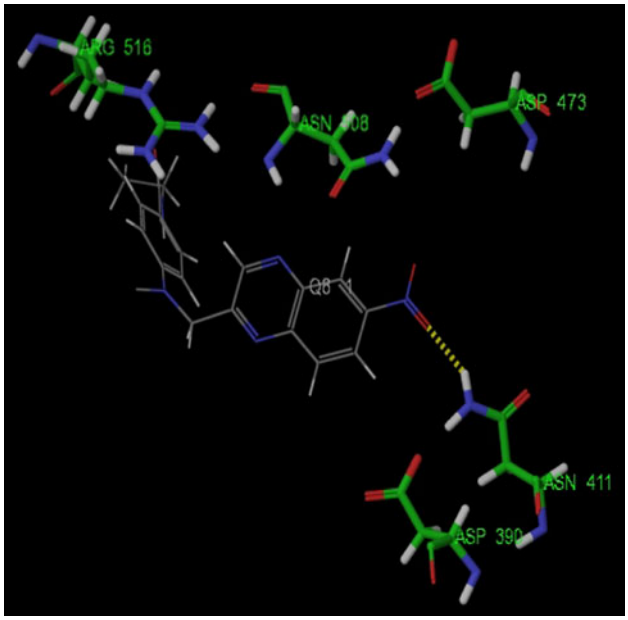



**Fig. 10** Image of compound 9 docked into the MTHF binding domain of MetS. Compound 9 made a hydrogen bond with asparagine 411

compound concentrations between 100 and 0.1 μM. The tested compounds were dissolved in DMSO. After 24 h, the cells were washed twice with Hanks solution and transferred to fresh $M + H^-$ medium (recovery). Cells were then incubated for a further 5 days at 37 °C before performing the MTT assay. Cell culture media was collected at appropriate times during recovery and stored at −80 °C. Appropriate control experiments were performed without the inhibitors.

MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) was used to assess the cytotoxicity of the tested compounds in cell culture. Following the above described treatments, media were recovered, and the cells were treated with fresh medium containing MTT (0.6 mg/mL). The cells were incubated for 4 h at 37 °C, the medium was removed and replaced with DMSO (150 μL) to dissolve the resulting crystals. The absorbance of this solution was recorded at 550 nm in a plate reader. The final DMSO concentration was 0.1 % and each plate contained the blank group (complete medium 200 μL/well and no cells) and the control group (complete medium 200 μL/well and untreated cells). After 96 h of exposure time, 20 μL of MTT solution (5 mg/mL) was added and incubated in the dark at 37 °C for 4 h. The supernatant was removed and formazan crystals were dissolved in DMSO (150 μL) (Lawrence *et al.*, 2006).

The results of the $IC_{50}$ values were listed down in Table 1.

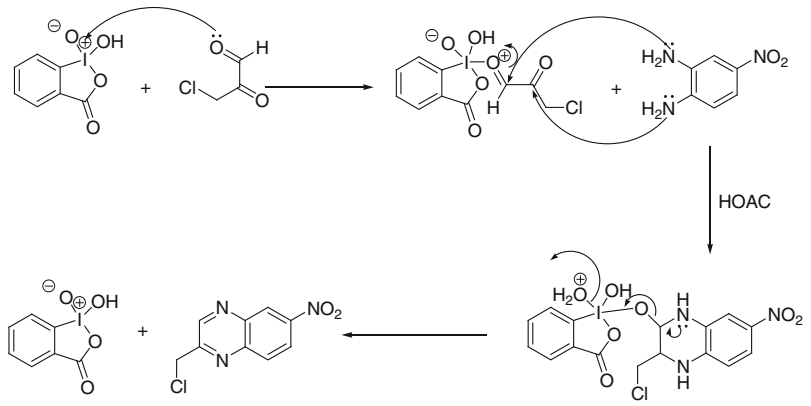



**Fig. 11** Mechanism of the synthesis of quinoxaline derivatives. The mechanism involves the activation of the 4-chlorobut-2-enal for the nucleophilic substitution reactions with IBX. The nucelophilic substitution started with the attack of the free lone pair nitrogen atoms of the 4-nitro-phenylen-1,2-diamine at the carbonyl carbon and β carbon atoms of 4-chlorobut-2-enal

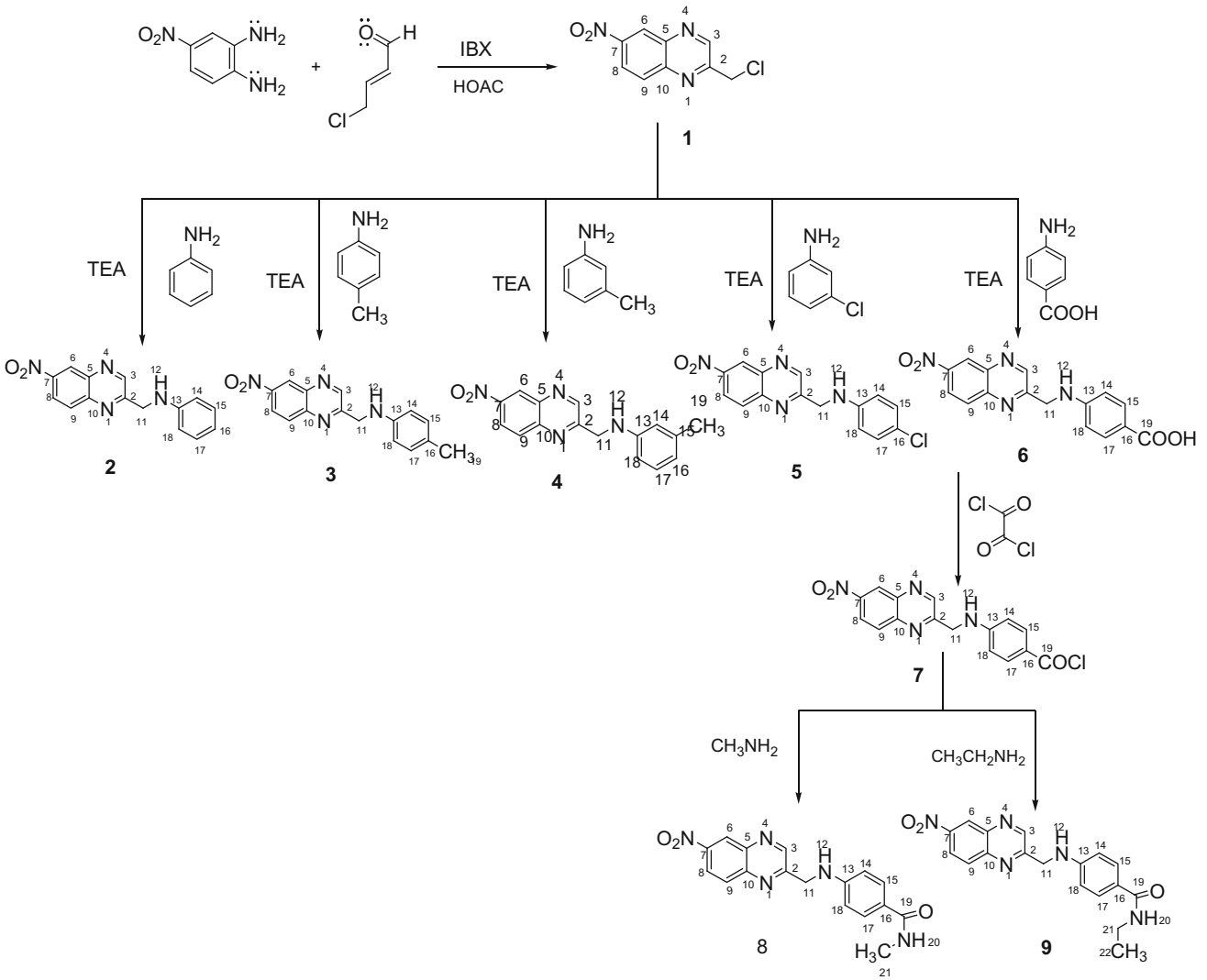
## Results

Compound 4-[{(6-nitro-quinoxalin-2-yl)methylamino} methyl]benzoic acid showed the lowest free energy of binding and the highest percentage of inhibition to the enzyme reaction free cell assay technique (89 %) and has got $IC_{50}$ values of 45 ± 9 and 53 ± 9 μM when screened against PC-3 and MCF-7 cells. Compound 2-chloromethyl-6-nitroquinoxaline showed the lowest percentage of inhibition of the enzyme reaction (55 %), high $IC_{50}$ values (70 ± 19 μM with PC-3 and 66 ± 19 μM with MCF-7), and the highest free binding energy −105.51 kJ/mol among the synthesized compounds in this article. Compounds *N*-[(6-nitroquinoxalin-2-yl)methyl]benzenamine, 4-methyl-*N*-[(6-nitroquinoxalin-2-yl)methyl]benzenamine, 3-methyl-*N*-[(6-nitroquinoxalin-2-yl)methyl]benzenamine, and 4-chloro-*N*-[(6-nitroquinoxalin-2-yl)methyl]benzenamine showed moderate percentage of inhibition of the enzyme reaction (65.9 %, 65 %, 45 %, and 45 %), $IC_{50}$ values (56 ± 8, 57 ± 14, 77 ± 10, and 70 ± 13 μM with PC-3 and 61 ± 6, 68 ± 14, 79 ± 14, and 75 ± 14 μM with MCF-7), and moderate free binding energies −105.51, −130.93, −132.91, −138.91, and −122.93 kJ/mol (Table 1).

## Discussion

Molecular modeling investigations were done on the MTHF receptor and the designed inhibitors were docked into the receptor on the same docking pose of the MTHF substrate (Fig. 2). Compounds 4-[(6-nitroquinoxalin-2-yl) methylamino)benzoic acid and 4-[(6-nitroquinoxalin-2-yl) methylamino)-*N*-methylbenzamide have been docked on the same pose orientation of the natural substrate of the enzyme. The root mean square deviations (RMSD) for the both compounds were 1.6 and 1.9 Å, respectively (Figs. 3, 4) (Danishpajooh *et al.*, 2001). This means that both compounds most likely will produce competitive inhibition and high concentration of the two compounds will displace MTHF from its receptor and, therefore, inhibit the enzyme reaction. The percentage inhibition, $IC_{50}$ values, and the free energy of the binding of the synthesized compounds were listed in Table 1.

Compound 1 made a hydrogen bond with arginine 516, and the quinoxaline nucleus docked vertically inside the pocket to avoid the van der Waals repulsive interactions (Fig. 5). The compounds achieved low free energy of binding of −105.51 kJ/mol and % inhibition of 55 %. This could be attributed to the steric hindrance caused by the rotamer methyl chloride moiety with the asparagine 411 and aspartic acid 390. The nitro group of the compound 2 made a hydrogen bond with amino group of asparagine 411 whereas the phenyl ring tended to point away from the polar argentine 516 positioned at the surface of the receptor and started to bend over to avoid the high energy barrier among the atoms of the ligand and the key residues of the enzyme cavity (Fig. 6). Compound 2 showed a moderate free energy of binding −130.93 kJ/mol and % inhibition of 65.9. Compounds 3, 4, and 5 tended to move away from the key amino acids of the receptor to avoid the polar-nonpolar repulsive interaction between the phenyl moiety and arginine 508 and 516 (Figs. 7, 8, 9) to avoid the steric clashes between the ligand and the amino acids of the receptor. These ligands had moderate free energy of binding −132.91, −138.91, and −122.93 kJ/mol, respectively, and % inhibition of 65, 45, and 45, respectively. $CH_3CH_2COPh$ moiety of compound 9 started to unfold and stretch out along the binding domain. This was attributed to the electrostatic interactions occurred between the amide group of the ligand and arginine 516




**Scheme 1** Mechanism of the synthesis of quinoxaline derivatives. The mechanism involves the activation of the 4-chlorobut-2-enal for the nucleophilic substitution reactions with IBX. The nucelophilic substitution started with the attack of the free lone pair nitrogen atoms of the 4-nitro-phenylen-1,2-diamine at the carbonyl carbon and beta carbon atoms of 4-chlorobut-2-enal. Subesequently nucleophilic substitution reaction occurred by the attack of various aromatic amines on the produced 2-Chloromethyl-6-nitroquinoxaline in the presence of triethyl amine to form the corresponding compounds 2–6. Oxylyl chloride will react with compound 6 to produce the corresponding aryl chloride compound 7 which in turn will react with aliphatic amine compounds methyl and theyl amines to produce final compounds 8 and 9

(Fig. 10). Therefore, the free energy of binding slightly increased and scored −145.89 kJ/mol and % inhibition increased to 70 %.

In this article we synthesized quinoxaline derivatives in the presence of IBX. Compound 1 was obtained by the condensation of 4-nitro-phenylen-1,2-diamine derivatives with 3-chloro-2-oxopropanal in the presence of IBX in acetic acid (Scheme 1). The reaction took longer time in the absence of IBX with many side products; therefore, IBX was used to reduce the side products and decrease the required reaction time, and facilitate the reaction conditions. We suggest that IBX can activate the carbonyl compounds as shown in Fig. 11.

## Conclusions

The results obtained from this study showed new generation of compounds that could probably be used as drugs that could be used in the treatment of breast and prostate carcinoma. Quinoxaline derivatives showed a meaningful inhibition of the methionine synthase reaction for both the somatic and cancer cells. Meanwhile, the somatic cells have the ability to use homocysteine as a precursor of biosynthase of methionine. The synthesized ligands showed from moderate to high inhibitory activity on the enzyme reaction with low concentration requirement to inhibit the growth of the cancer cells.

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## Appendix: The free energy of binding

$$\Delta E_{\text{Bind}} = \Delta E_{\text{Complex}} - (\Delta E_{\text{Receptor}} + \Delta E_{\text{Ligand}}) \quad (1)$$

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