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Screening of Quinoline, 1,3-Benzoxazine, and 1,3-Oxazine-based Small Molecules Against Isolated Methionyl-tRNA Synthetase and A549 and HCT116 Cancer Cells Including an In Silico Binding Mode Analysis

Hanumantharayappa Bharathkumar,^{a,#} Chakrabhavi Dhananjaya Mohan,^{b,#} Shobith Rangappa,^c Taehee Kang,^d Keerthy H K,^a Julian E. Fuchs,^e Nam Hoon Kwon,^d Andreas Bender,^e Sunghoon Kim,^{d,f} Basappa,^{a,*} Kanchugarakoppal S Rangappa,^{b,*}

^aLaboratory of Chemical Biology, Department of Chemistry, Bangalore University, Palace Road, Bangalore 560 001, India

^bDepartment of Studies in Chemistry, University of Mysore, Mysore 570 006, India

^cFrontier Research Center for Post-genome Science and Technology Hokkaido University, Japan

^dMedicinal Bioconvergence Research Center, Seoul National University, Seoul 151-742, Korea

^eCentre for Molecular Informatics, Department of Chemistry, University of Cambridge, Lensfield Road, CB2 1EW, Cambridge, United Kingdom

^fWCU Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Suwon 443-270, Korea.

[#]Contributed Equally

Abstract

Elevated activity of methionyl-tRNA synthetase (MRS) in many cancers renders it a possible drug target in this disease area, as well as in a series of parasitic diseases. In the present work, we report the synthesis and *in vitro* screening of a library of 1,3-oxazines, benzoxazines and quinoline scaffolds against human MRS. Among the compounds tested, 2-(2-butyl-4-chloro-1-(4-phenoxybenzyl)-1H-imidazol-5-yl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecane (compound 21) and 2-(2-butyl-4-chloro-1-(4-nitrobenzyl)-1H-imidazol-5-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine (Compound 8) were found to be potent inhibitors of MRS. Additionally, those compounds significantly suppressed the proliferation of A549 and HCT116 cells with an IC_{50} value of 28.4, 17.7 and 41.9, 19.8 μ M respectively. Molecular docking studies suggested that the ligand binding orientation overlaps with the original positions of both methionine and adenosine of MRS. This suggests the binding of compound 21 against MRS, which might lead to the inhibitory activity towards cancer cells.

Introduction

Aminoacyl-tRNA synthetases (ARS) are a group of enzymes that transfer a specific amino acid to cognate tRNA to form aminoacyl-tRNA.¹ The amino acylated-tRNA then carries the respective amino acid to the site of protein synthesis to begin translation at the initiation codon (or to continue this process).² The initiation codon codes for methionine, which is hence the first amino acid added during protein synthesis. Methionine specifically is esterified to tRNA^{met} by methionyl-tRNA synthetase (MRS), and elevated activity of MRS is reported in human cancers.³⁻⁵ In cancer cells, targeting translational initiation is considered as one of the effective strategy to inhibit the cell survival, proliferation and metastasis.⁶ Selective inhibition of human MRS hence results in blockade of early events of protein synthesis, and thereby termination of global translation.⁷ The human MRS up-regulation in cancer renders it as a unique target to design inhibitors, thereby inhibiting its functional properties.

Research in the previous decades explored numerous scaffolds including quinolines, pyrrolines and chloramphenicol derivatives as inhibitors of bacterial MRS, and most of them displayed high specificity towards bacterial MRS and failed in inhibiting human MRS.^{8, 9} Quinoline derivatives in particular have been extensively presented as inhibitors of bacterial MRS, and more recently the quinoline-4-one based small molecule REP8839 was reported as a novel inhibitor of MRS in methicillin-resistant *Staphylococcus aureus* as well as *Streptococcus pyogenes*.¹⁰ Furthermore, benzoxaborole derivatives were shown to have significant leucyl-tRNA inhibitory activity.¹¹ Overall, these studies indicate that many quinoline-, oxazine- and benzoxazine-based small molecules could be good candidates for the development of potent inhibitors against human as well as bacterial MRS.

We previously reported novel synthetic routes for the preparation of quinoline, benzoxazine and oxazine scaffolds and their derivatives.¹²⁻¹⁵ Herein, we report the design, synthesis, and biological evaluation of quinolines, benzoxazines, and oxazine derivatives against human methionyl-tRNA synthetase as well as cancer cell lines, including an analysis of putative binding modes against the target enzyme.

Results and Discussion

Chemistry

Since oxazine-based compounds have been reported to have good antioncogenic activity in hepatocellular carcinoma and osteosarcoma model, we here attempted to prepare newer oxazines by reacting 1-(2-amino-1-(4-methoxyphenyl)-ethyl)-cyclohexanol with various aldehydes in the presence of potassium carbonate in methanol media (Scheme 1). Further, 1,3-benzoxazines were prepared by reacting 2-amino benzyl alcohol with different aldehydes in the presence of chloroacetic acid in methanolic media (Scheme 2). Quinolines were prepared using 2-amino benzyl alcohol with various aldehydes in T3P (propylphosphonic anhydride) catalyzed reaction using Microwave Irradiation (Monowave G10 apparatus) (Scheme 3). The compounds obtained were characterized by melting point, ¹H NMR, ¹³C NMR, and mass spectral analysis. Detailed chemical characterizations of the newly synthesized compounds are provided in the Materials and Methods section.

In vitro screening of the library of small molecules against human MRS

Initially all the synthesized compounds were evaluated for their *in vitro* inhibitory activity against human MRS at the concentration of 100 μM. Among the newly synthesized structures, compounds **8**, **19**, **21**, **23**, **26** and **29** reduced aminoacylation activity of MRS over 60 % while compound **1** displayed minimal or no inhibitory activity (see **Figure 1**) and the MRS

activity was expressed based on the radioactivity (CPM: count per minute) of ^{35}S -Met charged tRNA. Methionine analog, Fmoc-DL-selenomethionine (Anaspec), was used as a positive control. The lead compounds were further evaluated at 25 μM and 100 μM and the results are expressed as relative inhibitory activity (see **Figure 2**). The relative inhibitory activity was calculated by converting CPM values of compound treated reaction into percentage inhibition with that of negative control (100 %). All the shortlisted compounds displayed a substantial decrease of MRS activity in a dose-dependent manner and compound **21**, which bears 1,3-oxazine ring attached to the substituted imidazole moiety was identified as the most potent inhibitor of human MRS. Further, we observed that, attachment of substituted imidazole moiety to oxazines and benzoxazines, haloarenes to quinolines contributed to the inhibition of MRS catalytic activity.

Compound 21 and compound 8 suppress the proliferation of A549 and HCT116 cancer cell lines

Given the activity of MRS in various cancers,^{16, 17} we next investigated the antiproliferative potential of the six lead compounds against A549 (lung carcinoma) and HCT116 (colon cancer) cells using the MTT assay.¹⁸ Paclitaxel was used as the reference drug. Among the tested compounds, compound **21** was found to be the most effective antiproliferative agent with the IC_{50} values of 28.4 and 17.7 μM , respectively, against the A549 and HCT116 cell lines, followed by compound **8** with IC_{50} values of 41.9 and 19.8 μM (see Table 1). The results of the cytotoxicity readout are hence closely correlated with in vitro MRS enzyme activity.

Molecular docking studies

Computational docking studies were performed next to understand the molecular interactions between the bioactive small molecules synthesized and tested earlier using

methionyl-tRNA synthetase (MRS) as the target structure (see Materials and Methods for details). We found consistent predicted binding modes for the oxazine series, placing the protonated nitrogen of the oxazines in vicinity of Asp296, thus forming charge-assisted hydrogen bonds and occupying the ribose position of the co-crystallized ligands. Furthermore, docked ligands were found to show major volume overlap with the original positions of both methionine and adenosine (**Figure 3**). Compound **21**, which has been found to be most active *in vitro* as well as exhibiting highest docking scores, is predicted to form π - π interactions via a methoxy phenyl group with Tyr15 and Trp253, that are also key anchor points for the substrate, methionine. A chloroimidazole moiety replaces the adenine base and a hydrophobic substituent forms additional π -stacking with His24, thus providing a hypothesis for the activity identified for this structure.

Experimental

Chemicals

All chemicals used were of analytical grade and purchased from Sigma Aldrich, and SRL, Mumbai (India). All IR spectra were obtained in a KBr disc on a Shimadzu FT-IR 157 Spectrometer. ^1H NMR spectra were recorded on a Bruker (400 MHz) spectrometer in CDCl_3 or DMSO-d_6 as solvent, using TMS as an internal standard, ^{13}C NMR spectra were recorded on a Bruker/Agilent (100 MHz) spectrometer and chemical shifts were expressed as δ ppm and abbreviations are assigned as, s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and J values are given in Hz. Mass spectra were determined on a Shimadzu LC-MS and ESI-MS, Elemental analyses were carried out using an Elemental Vario Cube CHNS Rapid Analyzer. Progress of the reaction was monitored by TLC pre-coated silica gel plates.

General procedure for the synthesis of 1,3-oxazines (1-7,9, 12-13 & 21): Synthesis of 1-(2-amino)-1-(4-methoxyphenylethyl) cyclohexanol (AA) - We initially prepared 1-[2-amino-1-(4-methoxy-phenyl)-ethyl]-cyclohexanolmonoacetate as described earlier¹⁵ and briefly summarized here. To a stirred solution of 1-(2-amino)-1-(4-methoxy-phenyl-ethyl)-cyclohexanolmonoacetate(1 eq) in methanol (10 mL) aldehydes (1 eq) and anhydrous potassium carbonate (2.5 eq) were added and the reaction mixture was stirred at room temperature for 9 h. After completion of the reaction, methanol was evaporated and water was added and extracted with ethyl acetate (15 mL). The combined organic layer was dried over anhydrous sodium sulphate. The crude solid was obtained on evaporation of the solvent under reduced pressure and recrystallized from hexane and ethyl acetate to furnish crystalline solid (Scheme 1)

Synthesis of 2-(2,6-difluorophenyl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecane (1) was carried out using the reported protocol.^{15, 19}

Synthesis of 2-(2-butyl-4-chloro-1H-imidazol-5-yl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecane (2): Compound **2** was obtained from AA (1 mmol), 2-butyl-4-chloro-1H-imidazole-5-carbaldehyde (1 mmol) and K₂CO₃ (2.5 mmol) as reddish brown crystalline solid, Yield: 89%, Melting point:108-110 °C; Elemental Analysis Calculated for C₂₃H₃₂ClN₃O₂: C, 66.09; H, 7.72; N, 10.05; found C, 66.14; H, 7.51; N, 10.13%; **IR v_{max} (KBr, cm⁻¹):** 3340, 2895, 1050; **¹H NMR** (CDCl₃, 400 MHz) δ: 7.85 (s, 1H), 7.15 (d, 2H), 6.8 (d, 2H), 4.9 (s, 1H), 4.2 (s,1H), 3.9 (m, 1H), 3.75 (s, 3H), 2.9 (dd, 2H), 2.6 (t, 2H), 1.8 -1.2 (m, 14H), 0.9 (t,3H); **¹³C NMR** (DMSO, 100 MHz)δ: 157.09, 147.73, 135.89, 134.55, 129.56, 120.81, 115.03, 88.62, 79.84, 55.60, 51.87, 48.12, 37.10, 36.93, 31.01, 28.20, 27.04, 22.97, 21.87, 20.95, 14.24; **Mass:** m/z found for C₂₃H₃₂ClN₃O₂: 418.2, 419.2.

Synthesis of 2-(1H-indol-3-yl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecane (3):
Compound 3 was obtained from AA (1 mmol), indole-3-carbaldehyde (1 mmol) and K_2CO_3 (2.5mmol) as brown crystalline solid, Yield: 90%; Melting point: 112-113⁰C; Elemental Analysis Calculated for $C_{24}H_{28}N_2O_2$: C, 76.56; H, 7.50; N, 07.44; found C, 76.65; H, 7.51; N, 07.23%; **IR** ν_{max} (KBr, cm^{-1}): 3260, 2910, 1150, **¹H NMR** (DMSO, 400 MHz) δ : 11.5 (s, 1H), 8.07-8.05 (d, $J=7.6$ Hz, 1H) 7.71 (s, 1H), 7.45-7.43(d, $J=8$ Hz, 1H), 7.24-7.17 (dd, $J_1=8$ Hz, $J_2= 8$ Hz, 4H), 7.08-7.11(t, $J=7.6$, 1H) 6.8 (d, $J=8.0$ Hz, 1H), 4.85(s, 1H), 4.22(s, 1H), 3.84-3.81(m, 1H), 3.73(s, 3H), 3.0-2.98(d, $J=5.6$, 1H), 1.71-1.11(m, 10H), **¹³C NMR** (DMSO, 100 MHz) δ : 157.56, 136.92, 133.13, 130.93, 130.53, 130.41, 124.79, 122.32, 121.29, 120.29, 114.25, 113.12, 112.85, 111.71, 89.25, 72.28, 55.87, 54.75, 41.58, 36.92, 34.10, 25.58, 21.61, 21.33; **Mass**: m/z found for $C_{24}H_{28}N_2O_2$: 377.4 ($[M+1]^+$)

Synthesis of 2-(4-bromophenyl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecane (4):
Compound 4 was obtained from AA (1 mmol), 4-Bromo benzaldehyde (1 mmol) and K_2CO_3 (2.5 mmol) as colorless crystalline solid, Yield: 90%; Melting point: 79-80 ⁰C; Elemental Analysis Calculated for $C_{22}H_{26}BrNO_2$: C, 63.46; H, 6.29; N, 03.36; found C, 63.34; H, 6.41; N, 03.25%. **IR** ν_{max} (KBr, cm^{-1}): 3290, 2910, 1210. **¹H NMR** ($CDCl_3$, 400 MHz) δ : 7.45-7.40(m, 4H), 7.08-7.06(d, $J=8.0$, 1H), 7.04-7.02(d, $J=8$ Hz, 1H), 6.78-6.76 (d, $J=8.8$ Hz, 1H), 6.73-6.71 (d, $J=8.8$ Hz, 1H), 5.34(s, 1H) 4.2(s, 1H), 3.73(s, 3H), 3.50(m, 1H), 3.01(d, 2H), 1.90-1.18(m, 10H). **¹³C NMR** (DMSO, 100MHz): 158.14, 133.04, 132.89, 129.83, 129.66, 128.29, 120.04, 115.97, 113.03, 112.89, 110.87, 86.90, 74.84, 57.02, 56.87, 44.22, 36.46, 25.16, 22.66, 19.23. **Mass**: m/z found for $C_{22}H_{26}BrNO_2$ 416.1, 418.1 ($[M+1]^+$).

Synthesis of 4'-((2-butyl-4-chloro-5-(5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecan-2-yl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (5): The compound 5 was obtained in two steps

Step 1: Preparation of 4'-((2-butyl-4-chloro-5-formyl-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile: was prepared using reported protocol.²⁰

Step 2: Preparation of 4'-((2-butyl-4-chloro-5-(5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecan-2-yl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile: This compound was obtained from AA (1 mmol), 4'-((2-butyl-4-chloro-5-formyl-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (1 mmol) and K₂CO₃ (2.5 mmol) in methanol as brown color crystalline solid, Yield: 88%; Melting point: 59-60 °C; Elemental Analysis Calculated for C₃₇H₄₁N₄ClO₂: C, 72.95; H, 6.78; N, 09.20; found C, 72.75; H, 6.51; N, 09.14%; **IR v_{max} (KBr, cm⁻¹):** 3265, 2914, 1170. **¹H NMR (DMSO, 400 MHz) δ:** 7.60-7.47(m,6H), 7.03-6.97(m,4H), 6.73-6.70(d, J=8.8,2H), 5.25(s, 1H), 5.10 (s,2H), 4.09 (s, 1H) 3.71(m,1H), 3.63(s,3H), 2.9(d,2H), 2.7(t,2H), 1.42-1.14(m,14H), 0.75-0.71(t,3H). **¹³C NMR (CDCl₃,100 MHz) δ:** 158.24, 151.90, 149.66, 144.99, 137.38, 137.30, 135.09, 133.95, 133.01,132.49, 130.66,130.10, 129.13, 127.81, 126.63, 122.30, 118.73,113.46, 111.35, 86.11, 73.38, 62.61, 56.30, 55.26, 47.96, 36.58, 35.29, 29.61,26.74, 25.84, 22.49, 22.01, 21.87,13.82; **Mass: m/z** found for C₃₇H₄₁N₄ClO₂: 610.2 ([M+1]⁺).

Synthesis of 5-(4-methoxyphenyl)-2-(2-methyl-1H-indol-3-yl)-1-oxa-3-azaspiro[5.5]undecane (6): Compound 6 was obtained from AA (1 mmol), 2-methyl indole-3-carbaldehyde (1 mmol) and K₂CO₃ (2.5 mmol) as colorless crystalline solid, Yield 95 %; Melting point: 158-160 °C; Elemental Analysis Calculated for C₂₅H₃₀N₂O₂: C, 76.89; H, 7.74; N, 07.17; found C, 76.65; H, 7.51; N, 07.14%. **IR v_{max} (KBr, cm⁻¹):** 3270, 3010, 1165, **¹H**

NMR (DMSO, 400 MHz) δ : 11.30(s, 1H), 7.91-7.89(d, J=8.0Hz, 1H), 7.26-7.25(d, J=7.6Hz, 1H), 7.18-7.16(d, J=8.0Hz, 2H), 7.04-6.98(m, 2H), 6.78-6.76(d, J=8.8Hz, 2H), 4.8(s, 1H), 4.2(s, 1H), 3.79(m, 1H), 3.71(s, 3H), 2.90(d, J=2.0Hz, 2H), 2.41(s, 3H), 1.64-1.21(m, 10H); **¹³C NMR** (CDCl₃, 100 MHz) δ : 158.27, 140.90, 135.60, 133.93, 130.72, 126.48, 122.52, 121.76, 120.48, 113.45, 110.71, 110.27, 86.04, 74.63, 63.54, 55.31, 47.01, 38.03, 34.35, 26.08, 22.22, 22.01, 12.30; **Mass**: m/z found for C₂₅H₃₀N₂O₂: 391.2 ([M+1]⁺).

Synthesis of 5-(4-methoxyphenyl)-2-(2-phenyl-1H-indol-3-yl)-1-oxa-3-azaspiro[5.5]undecane (7) :

Compound 7 was obtained from AA (1 mmol), 2-phenyl indole-3-carbaldehyde (1 mmol) and K₂CO₃ (2.5 mmol) as colorless crystalline solid, Yield: 93%; Melting point: 80-82 °C; Elemental Analysis Calculated for C₃₀H₃₂N₂O₂: C, 79.61; H, 7.13; N, 6.19; found C, 79.65; H, 7.01; N, 6.14% ; **IR** ν_{max} (KBr, cm⁻¹): 3310, 2890, 1170, **¹H NMR** (DMSO, 400 MHz) δ : 11.7(s, 1H,), 8.1(d, 2H), 7.6-7.40(m, 2H), 7.4-7.2(m, 3H), 7.2-7.0(m, 4H), 6.8(d, 2H), 4.5(s, 1H), 4.2(s, 1H), 3.8(m, 1H), 3.7(s, 3H), 2.9(d, 2H), 1.7-1.1(m, 10H), **¹³C NMR** (CDCl₃, 100MHz) δ : 158.24, 133.88, 131.41, 130.88, 129.39, 129.09, 129.02, 126.61, 123.73, 122.39, 122.26, 113.79, 113.44, 111.25, 111.05, 86.92, 74.34, 55.32, 50.91, 46.70, 37.97, 26.09, 22.26, 22.03. **Mass**: m/z found for C₃₀H₃₂N₂O₂: 453.3 ([M+1]⁺).

Synthesis of 4'-((3-(5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecan-2-yl)indolin-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (9): Compound 9 was prepared using the reported protocol.¹⁹

Synthesis of 4-(5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecan-2-yl)-N,N-dimethylaniline (12): Compound 12 was obtained from AA (1 mmol), 4-(dimethylamino) benzaldehyde (1 mmol) and K₂CO₃ (2.5 mmol) as brown color crystalline solid, Yield: 92%;

Melting point: 120-121 °C; Elemental Analysis Calculated for C₂₄H₃₄N₂O₂: C, 75.75; H, 8.48; N, 07.36; found C, 75.65; H, 8.51; N, 07.23%. **IR v_{max} (KBr, cm⁻¹):** 3280, 2866, 1118, **¹H NMR** (CDCl₃, 400 MHz) δ: 7.51-7.49(d, J=8.0Hz, 2H), 7.11-7.09(d, J=8.0Hz, 2H), 6.73-6.71(d, J=8Hz, 2H), 6.60-6.58(d, J=8.4Hz, 2H), 5.30(s,1H), 4.2(s,1H), 3.98-3.94(m,1H), 3.73(s, 3H), 2.94(s,6H), 2.90-2.88(d, J=8Hz, 2H), 1.77-1.28(m, 10H), **¹³C NMR** (CDCl₃, 100 MHz) δ: 158.01, 150.41, 135.50, 130.45, 130.10, 128.76, 114.11, 113.90, 112.98, 91.24, 79.21, 56.30, 50.97, 47.28, 41.45, 37.10, 26.56, 22.54, 22.34. **Mass:** m/z found for C₂₄H₃₄N₂O₂: 381.2 ([M+1]⁺).

Synthesis of 3-(5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecan-2-yl)-4H-chromen-4-one (13): Compound 13 was obtained from AA (1 mmol), 4-oxo-4H-chromene-3-carbaldehyde (1 mmol) and K₂CO₃ (2.5 mmol) as yellow color crystalline solid, Yield 91%; Melting point: 59-60 °C; Elemental Analysis Calculated for C₂₅H₂₇NO₄: C, 74.05; H, 6.71; N, 03.45; found C, 73.95; H, 6.51; N, 03.34 %. **IR v_{max} (KBr, cm⁻¹):** 3280, 2945, 1095, **¹H NMR** (DMSO, 400 MHz) δ: 7.66 (s, 1H), 7.2-6.6(m, 8H), 5.5(s, 1H), 4.1(s, 1H), 3.75(s, 3H), 3.5(m, 1H), 2.8(m, 2H), 1.7-0.9(m, 10H); **¹³C NMR** (CDCl₃, 100 MHz) δ: 191.05, 157.52, 157.30, 156.01, 133.50, 132.45, 131.12, 130.78, 124.53, 123.42, 118.78, 117.35, 113.15, 112.88, 86.12, 75.21, 56.34, 54.32, 47.13, 37.13, 36.40, 25.39, 21.21, 20.30; **Mass:** m/z found for C₂₅H₂₇NO₄ 406.2 ([M+1]⁺).

Synthesis of 2-(2-butyl-4-chloro-1-(4-phenoxybenzyl)-1H-imidazol-5-yl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecane (21): Compound 21 was obtained in two steps.

Step 1: Preparation of 2-butyl-4-chloro-1-(4-phenoxybenzyl)-1H-imidazole-5-carbaldehyde: This compound was obtained by using 2-butyl-4-chloro-1H-imidazole-5-carbaldehyde (1 mmol), 1-(bromomethyl)-4-phenoxybenzene (1.2 mmol), potassium carbonate (2.5 mmol), and DMF (8 mL) as a solvent and stirring for 14 h at room temperature.

Step 2: Preparation of 2-(2-butyl-4-chloro-1-(4-phenoxybenzyl)-1H-imidazol-5-yl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecane: This compound was obtained from AA (1 mmol), **2-butyl-4-chloro-1-(4-phenoxybenzyl)-1H-imidazole-5-carbaldehyde** (1 mmol), and K_2CO_3 (2.5 mmol) in methanol as brown crystalline solid, Yield: 84%; Melting point: 55-57 °C; Elemental Analysis Calculated for $C_{36}H_{42}N_3ClO_3$: C, 72.05; H, 7.05; N, 07.00; found C, 71.95; H, 6.91; N, 06.94%. **IR v_{max} (KBr, cm⁻¹):** 3290, 2920, 1150; **¹H NMR** ($CDCl_3$, 400 MHz) δ : 7.4-6.6(m,13H), 5.4(s, 1H), 5.3(s, 2H), 4.1(s, 1H) 3.75(s, 3H), 3.3(m,1H), 2.60(t,2H), 2.5(d,2H),1.7-1.2(m,17H); **¹³C NMR** (100 MHz, DMSO-d₆) δ : 157.90, 157.10, 154.43, 147.63, 140.24, 134.70, 130.78, 129.10, 128.55, 127.93, 122.12, 121.11, 119.23, 115.31, 87.24, 80.46, 56.10, 51.62, 47.65, 36.83, 30.91, 26.43, 25.80, 22.91, 22.40, 14.25; **Mass:** m/z found for $C_{36}H_{42}N_3ClO_3$: 600.4 ($[M+1]^+$).

Synthesis of 2-(2-butyl-4-chloro-1-(4-nitrobenzyl)-1H-imidazol-5-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine(8): Compound 8 was obtained in two steps.

Step 1: Preparation of 2-butyl-4-chloro-1-(4-nitrobenzyl)-1H-imidazole-5-carbaldehyde : This compound was obtained by using 2-butyl-4-chloro-1H-imidazole-5-carbaldehyde (1 mmol), 1-(bromomethyl)-4-nitrobenzene (1.2 mmol), potassium carbonate(2.5 mmol), and DMF (8 mL) as a solvent and stirring for 14 h at room temperature.

Step 2: Preparation of 2-(2-butyl-4-chloro-1-(4-nitrobenzyl)-1H-imidazol-5-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine : This compound was obtained from 2-amino benzyl alcohol (1 mmol), **2-butyl-4-chloro-1-(4-nitrobenzyl)-1H-imidazole-5-carbaldehyde** (1 mmol) and chloro acetic acid (2.5 mmol) in methanol at room temperature as a brown crystalline solid, Yield 81 %; Melting point 53-55 °C; Elemental Analysis Calculated for $C_{22}H_{23}ClN_4O_3$: C, 61.90; H, 5.43; N, 13.12; found C, 61.84; H, 5.41; N, 13.10%; **¹H NMR** (DMSO, 400 MHz) δ : 8.20-8.18(d,

J=8.4Hz, 2H), 7.20-7.13(m, 6H), 5.48(s, 1H), 4.99(s, 2H), 4.60(s, 2H), 4.24(s, 1H), 2.67-2.63(t, J=8Hz, 2H), 1.34-1.24(m, 2H), 0.89-0.85(t, J=7.2Hz, 3H); ^{13}C NMR (DMSO- d_6 , 100 MHz,) δ : 147.81, 144.74, 143.21, 140.75, 135.21, 128.72, 128.01, 126.71, 123.10, 122.05, 120.95, 110.96, 92.30, 67.31, 46.69, 31.21, 26.31, 22.85, 14.68. **Mass:** m/z found $\text{C}_{22}\text{H}_{23}\text{ClN}_4\text{O}_3$: 428.14 ($[\text{M}+1]^+$).

Synthesis of 2-(2-butyl-4-chloro-1H-imidazol-5-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine (10), 7-chloro-2-(1H-indol-3-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine (11), 3-(2,4-dihydro-1H-benzo[d][1,3]oxazin-2-yl)-4H-chromen-4-one (14), 2-(1H-indol-3-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine (15), 3-(6-methyl-2,4-dihydro-1H-benzo[d][1,3]oxazin-2-yl)-4H-chromen-4-one (16), 4'-((3-(2,4-dihydro-1H-benzo[d][1,3]oxazin-2-yl)-1H-indol-1-yl)methyl)-[1,1'-biphenyl]-2-carbonitrile (17), 6-chloro-2-(2-phenyl-1H-indol-3-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine (18), 3-(6-chloro-2,4-dihydro-1H-benzo[d][1,3]oxazin-2-yl)-4H-chromen-4-one (19), 6-methyl-2-(2-methyl-1H-indol-3-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine (20), 6-methyl-2-(2-phenyl-1H-indol-3-yl)-2,4-dihydro-1H-benzo[d][1,3]oxazine (22), 4-(2,4-dihydro-1H-benzo[d][1,3]oxazin-2-yl)phenol (23), and 4-(7-chloro-2,4-dihydro-1H-benzo[d][1,3]oxazin-2-yl)phenol (24) were prepared using the reported protocol.¹⁴

Synthesis of 2-(quinolin-2-yl)phenol (25), 2-(2-bromophenyl)-6-methylquinoline (26), 2-(2,4-dichlorophenyl)Quinoline (27), 4-(6-chloroquinolin-2-yl)benzenamine (28), and 2-(2,4-dichlorophenyl)-6-methylquinoline (29) were prepared using the reported protocol.¹³

Pharmacology

Human methionyl-tRNA synthetase inhibition assay. MRS was co-expressed with AIMP3 (aminoacyl-tRNA synthetase-interacting multifunctional protein 3), which does not affect the

catalytic activity of MRS *in vitro*, in *Escherichia Coli* BL21(DE3) to increase protein stability and solubility and purified using ProBond Resin (Invitrogen).²¹ MRS was eluted in the presence of 200 mM imidazole (pH 6.0) and dialyzed with PBS containing 20% (vol/vol) glycerol. Aminoacylation activity of MRS was measured at 37°C in reaction buffer (30 mM HEPES, pH 7.4, 100 mM potassium acetate, 10 mM magnesium acetate, 2mM ATP, 100 µg/mL tRNAⁱ Met, and 25 µCi [35S] Methionine (1000 Ci/mmol; Izotop)) in the presence of 25 µM or 100 µM inhibitor. Aminoacylation reactions were quenched on 3 MM filter paper prewetted with 5% trichloroacetic acid containing 1 mM Methionine. After washing with 5% trichloroacetic acid and drying, radioactivity was detected by liquid scintillation counter. Fmoc-DL-selenomethionine was used as positive control for human methionyl tRNA inhibitory studies.

MTT assay. The antiproliferative effect of the compounds synthesized against HCT116 (colon cancer) and A549 (lung cancer) cells was determined by the MTT dye uptake method as described previously.^{22, 23} Briefly, cancer cells (2.5×10^4 /mL) were incubated in triplicate in a 96-well plate, in the presence of varying compound concentrations at a volume of 0.2 mL, for different time intervals at 37 °C. Thereafter, 20 µl MTT solution (5 mg/mL in PBS) was added to each well. After 2 h incubation at 37 °C, a 0.1 mL lysis buffer (20% SDS, 50% dimethylformamide) was added; incubation was performed for 1 h at 37 °C, and the optical density (OD) at 570 nm was measured by plate reader.

Molecular docking studies. Computational docking studies were performed to investigate molecular interactions between the library of the quinoline, oxazine and benzoxazine ligands and MRS. Therefore, we prepared the whole set of 34 ligands for docking (addition of explicit hydrogens and protonation for pH=7) using MOE²⁴ and selected the co-crystal structure of MRS with methionine and adenosine (PDB: 1PG2) as the target structure.²⁵ The protein structure was

prepared using protonate3D²⁶ whilst the co-crystallized ligand as well as resolved water molecules were discarded. We selected the position of the C5' atom of adenosine as the cavity centre and used GOLD²⁷ for docking in a radius of 10Å around the centre.

Statistical Analysis. The mean values are expressed \pm S.E. for control and experimental samples and each experiment was repeated a minimum of two times. Statistical significance of data was determined by applying Student's t test using the Prism 5 software (GraphPad Software).

Conclusion

Elevated levels of protein synthesis in cancer cells serve as an attractive target for designing of anticancer agents. Since translation process involves the incorporation of methionine as first amino acid of all the polypeptides during protein synthesis and inhibition of esterification of methionine to tRNA^{Met} by MRS results in collapse of protein synthesis. In order to provide suitable small molecules to inhibit this process, in the current study we report the synthesis and biological evaluation of a library of small molecules against human MRS, which led to the identification of five bioactive molecules. Among the newly synthesized compounds, compound **21** and compound **8** were presented as the most potent inhibitors of human MRS, and, on the other hand, both compounds displayed cytotoxicity against cancer cell lines at relatively sub-micromolar concentrations.

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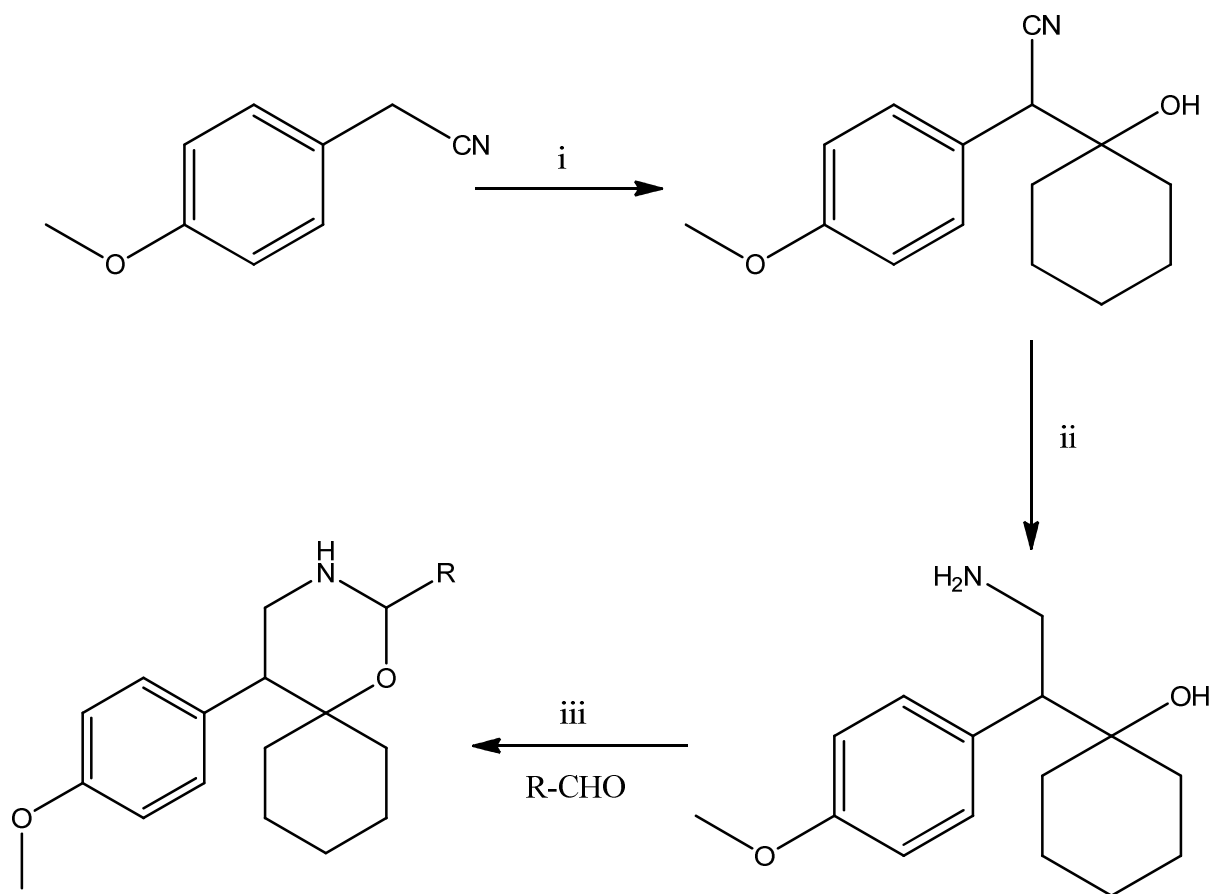
Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare (HI13C21480301[N.H.K]) and by the Global Frontier program (NRF-M3A6A4-2010-0029785 [S.K.])

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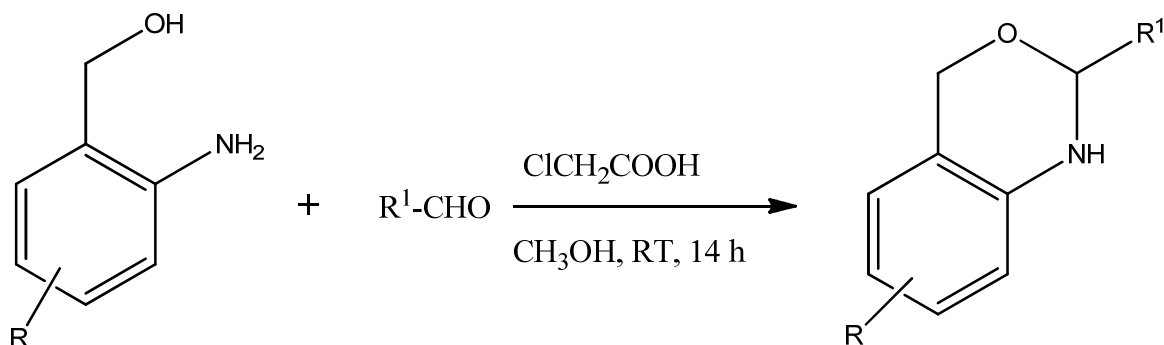
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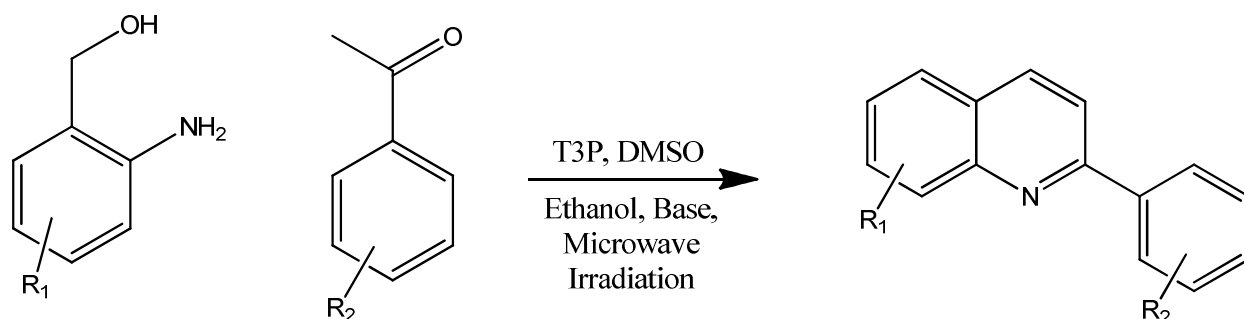
Schemes



Scheme 1: schematic representation for the synthesis of 1,3-oxazines: (i) cyclohexanone, NaOH, Bu₄NBr, water–MeOH, RT, 15 h, 96%; (ii) Raney-Ni, H₂ (10 atm), anhydrous NH₃, MeOH, 35–40 °C, 3 h. (iii) R-CHO, anhydrous K₂CO₃/CH₃OH, RT, 9 h, (Yield: 85–95%)



Scheme 2: Schematic representation for the synthesis of benzoxazines (Yield: 85–96%).



Scheme 3: Schematic representation for the synthesis of quinolines (Yield: 86%).

Figure Legends

Figure 1: Screening of new compounds (1-34) against the activity of human methionyl-tRNA synthetase at 100 μ M. Data are represented as mean \pm S.E. (n = 3). PC, positive control, Fmoc-DL-selenomethionine; *, P < 0.05; **, P < 0.01.

Figure 2: The lead molecules, which exhibited inhibitory activity against human methionyl-tRNA synthetase at 100 μ M were chosen and evaluated at 25 μ M and 100 μ M.

Figure3: Predicted molecular interactions of the most active compound **21** and methionyl-tRNA synthetase (from PDB 1PG2). Left: Native contacts of the ligands (elemental colours with carbon in grey) and MRS (green cartoon). Side-chains of key amino acids (Asp296, Trp253, Tyr15, His24) are highlighted as lines. Right: predicted binding mode for compound **21**, that shows major volume overlap with the native ligands. The central oxazine moiety is interacting with Asp-296, whereas substituents form multiple hydrophobic contacts and π - π interactions.

Table 1: Cytotoxic studies of lead compounds (against HCT116 and A549 cells) that targets human MRS.

Lead Compounds	HCT116	A549
	IC ₅₀ (μM)	IC ₅₀ (μM)
Compound 8	19.8 ± 4.9	41.9 ± 5.7
Compound 19	>50	>50
Compound 21	17.7 ± 3.9	28.4 ± 6.1
Compound 23	49.1 ± 7.8	>50
Compound 26	41.3 ± 4.6	>50
Compound 29	40.4 ± 5.5	>50
Paclitaxel	0.0054	0.0046

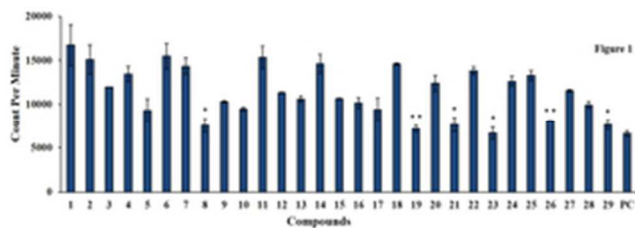
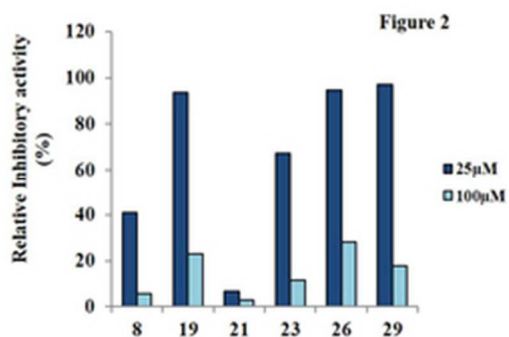
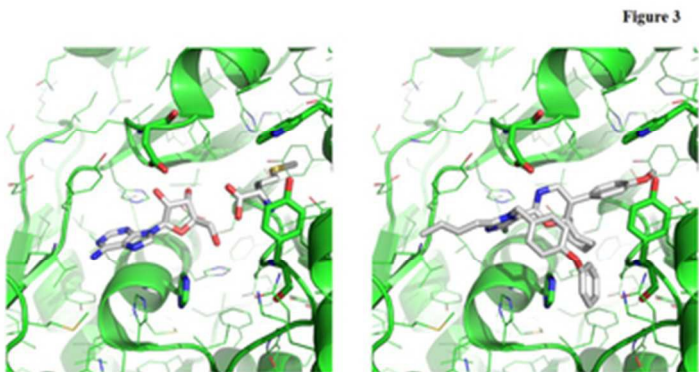


Figure 1: Screening of new compounds (1-34) against the activity of human methionyl-tRNA synthetase at 100 μ M. Data are represented as mean \pm S.E. (n = 3). PC, positive control, Fmoc-DL-selenomethionine; *, P < 0.05; **, P < 0.01.

27x9mm (300 x 300 DPI)



The lead molecules, which exhibited inhibitory activity against human methionyl-tRNA synthetase at 100 μ M were chosen and evaluated at 25 μ M and 100 μ M.
22x14mm (300 x 300 DPI)



30x16mm (300 x 300 DPI)