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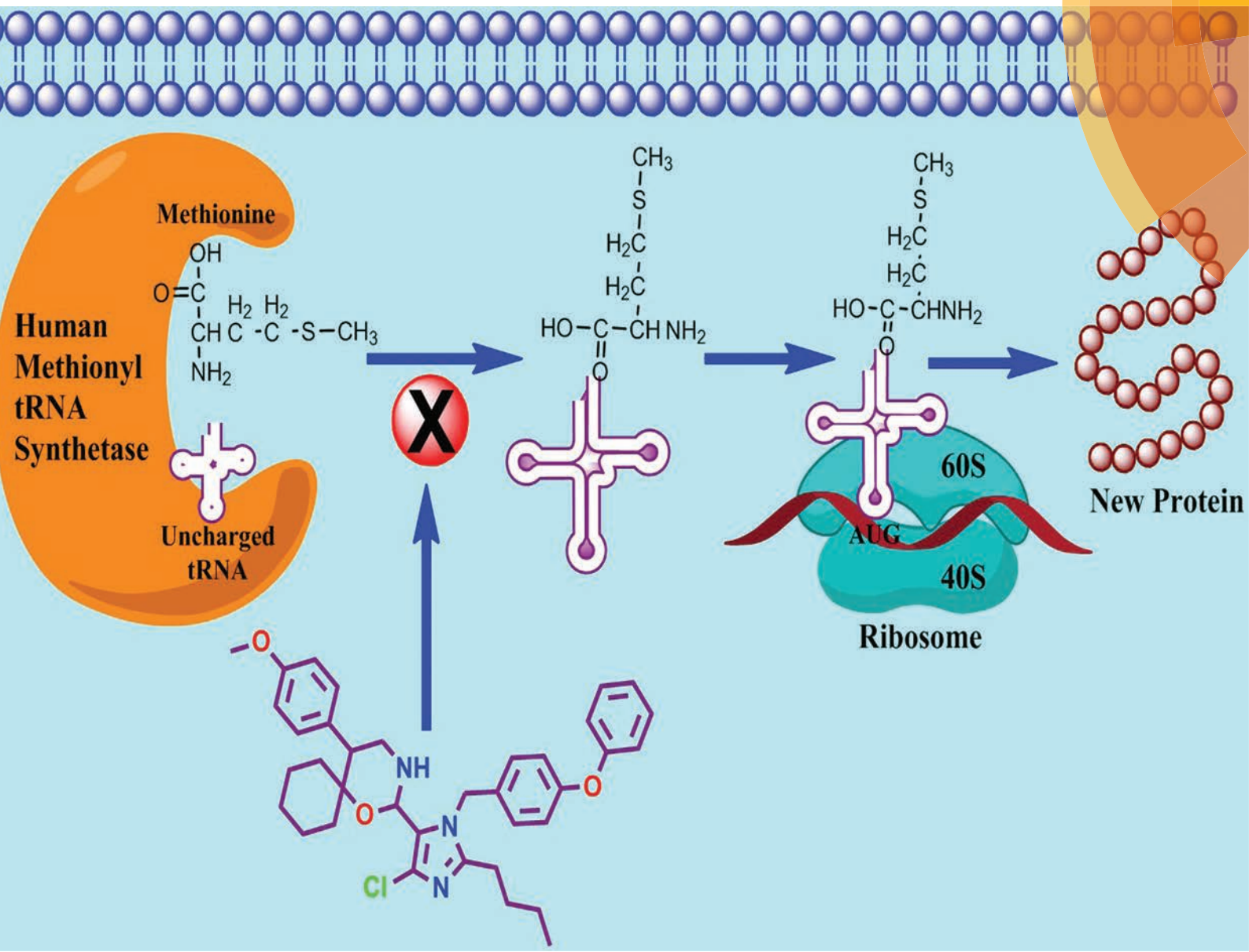

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PAPER

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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†

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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)-1*H*-imidazol-5-yl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecane (compound **21**) and 2-(2-butyl-4-chloro-1-(4-nitrobenzyl)-1*H*-imidazol-5-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine (compound **8**) were found to be potent inhibitors of MRS. Additionally, these compounds significantly suppressed the proliferation of A549 and HCT116 cells with IC_{50} values of 28.4, 17.7, 41.9, and 19.8 μM 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 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 strategies to inhibit 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

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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.

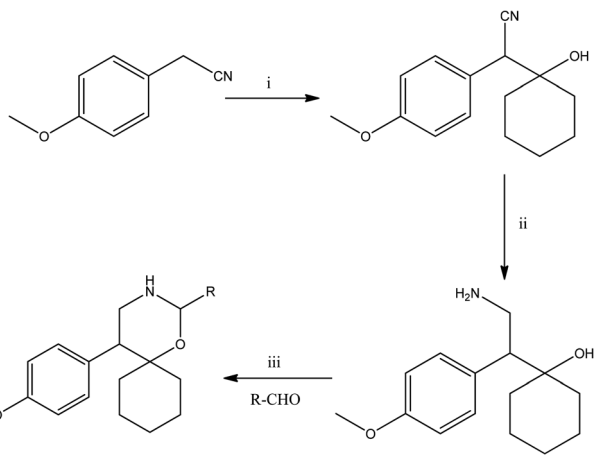
We previously reported novel synthetic routes for the preparation of quinoline, benzoxazine and oxazine scaffolds and their derivatives.[12–15] Herein, we report the design, synthesis, and biological evaluation of quinolines, benzoxazines, and oxazine derivatives against human methionyl-tRNA synthetase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

Results and discussion

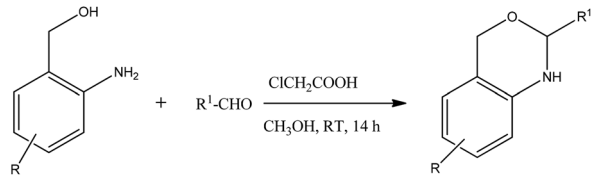
Chemistry

Since oxazine-based compounds have been reported to have good antioncogenic activity in hepatocellular carcinoma and osteosarcoma models, here we 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 reactions using microwave irradiation (Monowave G10 apparatus) (Scheme 3). The compounds obtained were characterized by melting point, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis. Detailed chemical characterization of the newly synthesized compounds is provided in the Experimental 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 Fig. 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 the relative inhibitory activity (see Fig. 2). The relative inhibitory activity was calculated by converting CPM values of the 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 a 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 the substituted imidazole moiety to oxazines and benzoxazines, and haloarenes to quinolines contributed to the inhibition of MRS catalytic activity.



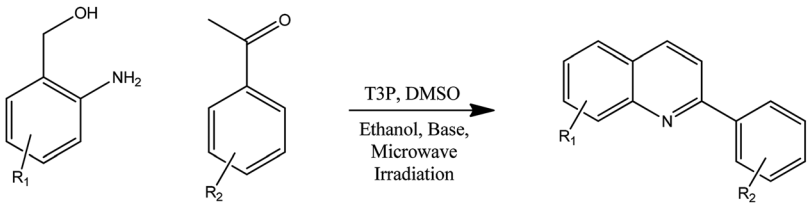
Scheme 1 Schematic representation for the synthesis of 1,3-oxazines: (i) cyclohexanone, NaOH, Bu_4NBr, water–MeOH, RT, 15 h, 96%; (ii) RANEY® Ni, H_2 (10 atm), anhydrous NH_3, MeOH, 35–40 °C, 3 h. (iii) R–CHO, anhydrous K_2CO_3/CH_3OH, RT, 9 h, (yield: 85–95%).



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

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 the 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 Experimental for details). We found consistent predicted binding modes for the oxazine series, placing the protonated nitrogen of the oxazines in the 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 a major volume overlap



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

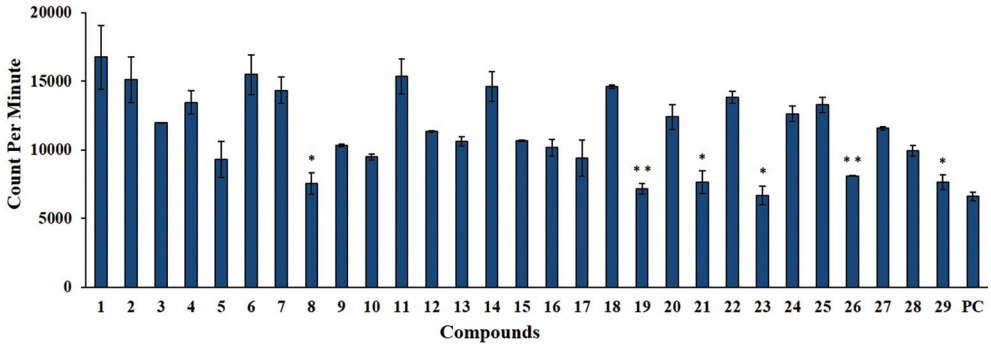


Fig. 1 Screening of new compounds (**1–29**) against the activity of human methionyl-tRNA synthetase at 100 μM. Data are represented as mean ± S.E. (n = 3). PC, positive control, Fmoc-DL-selenomethionine; *, $P < 0.05$; **, $P < 0.01$.

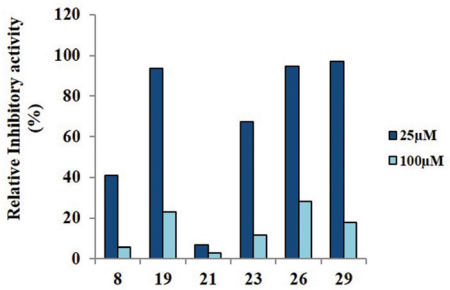


Fig. 2 The lead molecules, which exhibited inhibitory activity against human methionyl-tRNA synthetase at 100 μM were chosen and evaluated at 25 μM and 100 μM.

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

	HCT116	A549
Lead compounds	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

with the original positions of both methionine and adenosine (Fig. 3). Compound **21**, which has been found to be the most active *in vitro* as well as exhibiting the 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 recorded in a KBr disc on a Shimadzu FT-IR 157 spectrometer. ^{1}H NMR spectra were recorded on a Bruker (400 MHz) spectrometer in $CDCl_3$ or DMSO-d_6 as a 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 recorded on a Shimadzu LC-MS and ESI-MS, and elemental analyses were carried out using an Elemental Vario Cube CHNS Rapid Analyzer. The progress of the reaction was monitored by TLC precoated silica gel plates.

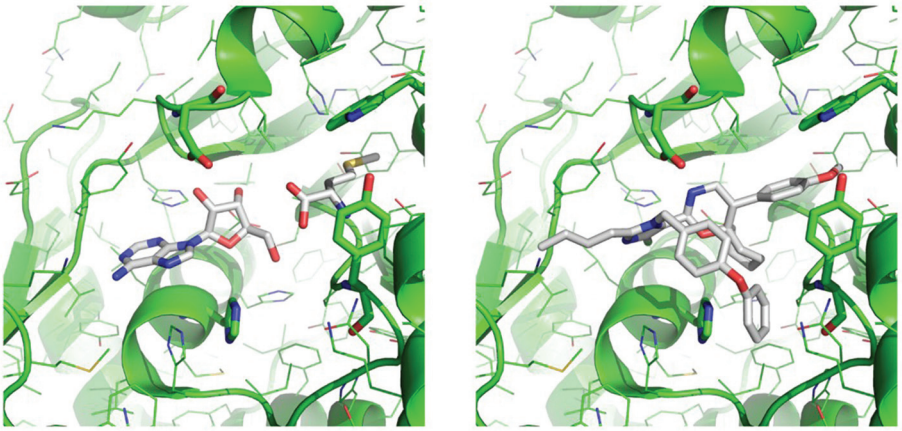


Fig. 3 Predicted molecular interactions of the most active compound **21** and methionyl-tRNA synthetase (from PDB 1PG2). Left: native contacts of the ligands (elemental colours with carbon in grey) and MRS (green cartoon). Side-chains of key amino acids (Asp296, Trp253, Tyr15, His24) are highlighted as lines. Right: predicted binding mode for compound **21**, which shows a major volume overlap with the native ligands. The central oxazine moiety is interacting with Asp-296, whereas substituents form multiple hydrophobic contacts and $\pi-\pi$ interactions.

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 earlier[15] 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 a 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-1*H*-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-1*H*-imidazole-5-carbaldehyde (1 mmol) and K_2CO_3 (2.5 mmol) as a reddish brown crystalline solid, yield: 89%, melting point: 108–110 °C; elemental analysis calculated for $C_{23}H_{32}ClN_3O_2$: C, 66.09; H, 7.72; N, 10.05; found C, 66.14; H, 7.51; N, 10.13%; IR ν_{max} (KBr, cm^{-1}): 3340, 2895, 1050; ^1H NMR ($CDCl_3$, 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); ^{13}C 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 $C_{23}H_{32}ClN_3O_2$: 418.2, 419.2.

Synthesis of 2-(1*H*-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 (2.5 mmol) as a brown crystalline solid, yield: 90%; melting point: 112–113 °C; 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), ^{13}C 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 a colorless crystalline solid, yield: 90%; melting point: 79–80 °C; 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). ^{13}C NMR (DMSO, 100 MHz): 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)-1*H*-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-1*H*-imidazol-1-yl)methyl)-[1,1′-biphenyl]-2-carbonitrile: was prepared using the 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)-1*H*-imidazol-1-yl)methyl)-[1,1′-biphenyl]-2-carbonitrile: this compound was obtained from AA (1 mmol), 4′-((2-butyl-4-chloro-5-formyl-1*H*-imidazol-1-yl)methyl)-[1,1′-biphenyl]-2-carbonitrile (1 mmol) and K_2CO_3 (2.5 mmol) in methanol as a brown crystalline solid, yield: 88%; melting point: 59–60 °C; elemental analysis calculated for $C_{37}H_{41}N_4ClO_2$: C, 72.95; H, 6.78; N, 09.20; found C, 72.75; H, 6.51; N, 09.14%; IR ν_{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). ^{13}C NMR ($CDCl_3$, 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 $C_{37}H_{41}N_4ClO_2$: 610.2 ($[M + 1]^+$).

Synthesis of 5-(4-methoxyphenyl)-2-(2-methyl-1*H*-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 K_2CO_3 (2.5 mmol) as a colorless crystalline solid, yield 95%; melting point: 158–160 °C; elemental analysis calculated for $C_{25}H_{30}N_2O_2$: C, 76.89; H, 7.74; N, 07.17; found C, 76.65; H, 7.51; N, 07.14%. IR ν_{max} (KBr, cm^{-1}): 3270, 3010, 1165, ^1H NMR (DMSO, 400 MHz) δ:11.30 (s, 1H), 7.91–7.89 (d, J = 8.0 Hz, 1H), 7.26–7.25 (d, J = 7.6 Hz, 1H), 7.18–7.16 (d, J = 8.0 Hz, 2H), 7.04–6.98 (m, 2H), 6.78–6.76 (d, J = 8.8 Hz, 2H), 4.8 (s, 1H), 4.2 (s, 1H), 3.79 (m, 1H), 3.71 (s, 3H), 2.90 (d, J = 2.0 Hz, 2H), 2.41 (s, 3H), 1.64–1.21 (m, 10H); ^{13}C NMR ($CDCl_3$, 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 $C_{25}H_{30}N_2O_2$: 391.2 ($[M + 1]^+$).

Synthesis of 5-(4-methoxyphenyl)-2-(2-phenyl-1*H*-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 K_2CO_3 (2.5 mmol) as a colorless crystalline solid, yield: 93%; melting point: 80–82 °C; elemental analysis calculated for $C_{30}H_{32}N_2O_2$: C, 79.61; H, 7.13; N, 06.19; found C, 79.65; H, 7.01; N, 06.14%; 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), ^{13}C NMR ($CDCl_3$, 100 MHz) 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 $C_{30}H_{32}N_2O_2$: 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 K_2CO_3 (2.5 mmol) as a brown crystalline solid, yield: 92%; melting point: 120–121 °C; elemental analysis calculated for $C_{24}H_{34}N_2O_2$: C, 75.75; H, 8.48; N, 07.36; found C, 75.65; H, 8.51; N, 07.23%. IR ν_{max} (KBr, cm^{-1}): 3280, 2866, 1118, ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.51–7.49 (d, J = 8.0 Hz, 2H), 7.11–7.09 (d, J = 8.0 Hz, 2H), 6.73–6.71 (d, J = 8 Hz, 2H), 6.60–6.58 (d, J = 8.4 Hz, 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 = 8 Hz, 2H), 1.77–1.28 (m, 10H), ^{13}C NMR ($CDCl_3$, 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 $C_{24}H_{34}N_2O_2$: 381.2 ($[M + 1]^+$).

Synthesis of 3-(5-(4-methoxyphenyl)-1-oxa-3-azaspiro[5.5]undecan-2-yl)-4*H*-chromen-4-one (13). Compound **13** was obtained from AA (1 mmol), 4-oxo-4*H*-chromene-3-carbaldehyde (1 mmol) and K_2CO_3 (2.5 mmol) as a yellow crystalline solid, yield 91%; melting point: 59–60 °C; elemental analysis calculated for $C_{25}H_{27}NO_4$: C, 74.05; H, 6.71; N, 03.45; found C, 73.95; H, 6.51; N, 03.34%. IR ν_{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); ^{13}C NMR ($CDCl_3$, 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 $C_{25}H_{27}NO_4$: 406.2 ($[M + 1]^+$).

Synthesis of 2-(2-butyl-4-chloro-1-(4-phenoxybenzyl)-1*H*-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)-1*H*-imidazole-5-carbaldehyde: this compound was obtained by using 2-butyl-4-chloro-1*H*-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)-1*H*-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)-1*H*-imidazole-5-carbaldehyde (1 mmol), and K_2CO_3 (2.5 mmol) in methanol as a 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 ν_{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); ^{13}C NMR (100 MHz, DMSO-d_6) 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)-1*H*-imidazol-5-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine (8). Compound **8** was obtained in two steps.

Step 1: Preparation of 2-butyl-4-chloro-1-(4-nitrobenzyl)-1*H*-imidazole-5-carbaldehyde: this compound was obtained by using 2-butyl-4-chloro-1*H*-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 by stirring for 14 h at room temperature.

Step 2: Preparation of 2-(2-butyl-4-chloro-1-(4-nitrobenzyl)-1*H*-imidazol-5-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine: this compound was obtained from 2-amino benzyl alcohol (1 mmol), 2-butyl-4-chloro-1-(4-nitrobenzyl)-1*H*-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%; ^{1}H NMR (DMSO, 400 MHz) δ: 8.20–8.18 (d, J = 8.4 Hz, 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 = 8 Hz, 2H), 1.34–1.24 (m, 2H), 0.89–0.85 (t, J = 7.2 Hz, 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 $C_{22}H_{23}ClN_4O_3$: 428.14 ($[M + 1]^+$).

Synthesis of 2-(2-butyl-4-chloro-1*H*-imidazol-5-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine (**10**), 7-chloro-2-(1*H*-indol-3-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine (**11**), 3-(2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-2-yl)-4*H*-chromen-4-one (**14**), 2-(1*H*-indol-3-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine (**15**), 3-(6-methyl-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-2-yl)-4*H*-chromen-4-one (**16**), 4′-((3-(2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-2-yl)-1*H*-indol-1-yl)-methyl)-[1,1′-biphenyl]-2-carbonitrile (**17**), 6-chloro-2-(2-phenyl-1*H*-indol-3-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine (**18**), 3-(6-chloro-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-2-yl)-4*H*-chromen-4-one (**19**), 6-methyl-2-(2-methyl-1*H*-indol-3-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine (**20**), 6-methyl-2-(2-phenyl-1*H*-indol-3-yl)-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazine (**22**), 4-(2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-2-yl)phenol (**23**), and 4-(7-chloro-2,4-dihydro-1*H*-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, 2 mM ATP, 100 μg mL^{-1} tRNAi Met, and 25 μCi [35S] methionine (1000 Ci mmol^{-1}; 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 using a liquid scintillation counter. Fmoc-DL-selenomethionine was used as a 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^{-1}) 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^{-1} 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 using a 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 29 ligands for docking (addition of explicit hydrogens and protonation for pH = 7) using MOE[24] 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 protonate3D[26] 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 GOLD[27] for docking with a radius of 10 Å around the centre.

Statistical analysis. The mean values are expressed ± 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 Prism 5 software (GraphPad Software).

Conclusion

Elevated levels of protein synthesis in cancer cells serve as an attractive target for developing anticancer agents. The translation process involves the incorporation of methionine as the first amino acid of all the polypeptides during protein synthesis and inhibition of esterification of methionine to $tRNA^{Met}$ by MRS results in the 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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