Electronic supplementary Information

Synthesis of tetrazole-isoxazoline hybrids as a new class of tubulin polymerization inhibitors

Ahmed Kamal,*a A. Viswanath,a M. Janaki Ramaiah,b J.N.S.R.C. Murty,a Farheen Sultana,a G. Ramakrishna,a Jaki R. Tamboli,a S.N.C.V.L. Pushpavalli,b Dhananjaya pal,b Chandan Kishor,b Anthony Addlagatta,*b Manika pal Bhadra*,b

aDivision of Organic Chemistry, bChemical Biology Laboratory,
CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India
E-mail: ahmedkamal@iict.res.in; Tel.: +91-40-27193157; Fax: +91-40-27193189

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General. (A) Chemistry.

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. $^1$H spectra were recorded on Bruker UXNMR/XWIN-NMR (300 MHz) or Inova Varian-VXR-unity (400, 500 MHz) instruments. Chemical shifts ($\delta$) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI$^+$ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. IR spectra (KBr) were measured with a Thermo Nicolet Nexus 670 Spectrometer ($\nu$ in cm$^{-1}$). Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

(B) Biology.

(a) Cell culture: Human lung cancer cell line A549 cells was purchased from American Type culture collection was maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen), supplemented with 2 mM glutamax (Invitrogen), 10 % fetal calf serum and 100 U/ml Pencillin and 100 µg/ml streptomycin sulfate (Sigma). The cell line was maintained at 37 °C in a humidified atmosphere containing 5 % CO$_2$ in the incubator.

(b) MTT assay: The anticancer activity of the compounds was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay. Around 1×10$^4$ cells/well were seeded in 100 µl DMEM (Dulbecco’s Modified Eagle’s Medium) supplemented with 10% FBS in each well of 96-well plates and were incubated for 24 h at 37 °C in a CO$_2$ incubator. After 24 h of incubation, cells were treated with the test compounds for 48 h. After the treatment, 10µl of MTT (5 mg/mL) was added to each well and the plates were further incubated for 4 h. After incubation, supernatant from each well was carefully removed and formazan crystals were dissolved in 100 µl of dimethyl sulfoxide (DMSO). Finally, the absorbance was measured at 570 nm. The MTT
assay was performed for all the test compounds in human tumor cells such as A549 and MDA-MB-231.

(c) Cell cycle analysis: $5 \times 10^5$ A549 cells were seeded in 60 mm dish and were allowed to grow for 24 h. Compounds 4a-l, CA-4 (1, positive control), 1 and 3 (starting materials) at 2 μM concentration were added to the culture media, and the cells were incubated for an additional 24 h. Cells were harvested with Trypsin-EDTA, fixed with ice-cold 70 % ethanol at 4 °C for 30 min, washed with PBS and incubated with 1mg/ml RNase A solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm for 5 min and further stained with 250 μL of DNA staining solution [10 mg of Propidium Iodide (PI), 0.1 mg of trisodium citrate, and 0.03 mL of Triton X-100 were dissolved in 100 mL of sterile MilliQ water at room temperature for 30 min in the dark]. The DNA contents of 20,000 events were measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit Software.

(d) Immunofluorescence: A549 cells were seeded on cover slips and treated with compound 4h, 4i, nocodazole (Noc), CA-4 (1), paclitaxel (Pac) at concentration of 2 μM for 24h. After treatment, cover slips were fixed with a paraformaldehyde solution (4% in 1X PBS) for 20 min at room temperature. Cell permeabilization was achieved by administration of a Triton X-100 solution (0.2 % in 1X PBS) for 5 min. Further the cover slips were kept overnight in 100% methanol at 4 °C. Subsequently, cover slips were blocked with a 1 % BSA solution for 60 min and then incubated with anti β-tubulin and γ-tubulin antibodies (1:100) at room temperature for 2 h. The slides were washed three times each of 5 min with PBST. Then cover slips were incubated with a FITC-conjugated anti-rabbit secondary antibody (Jackson Immuno Research Laboratories Inc., Pennsylvania, USA) for 1 h and cover slips were washed three times with PBST solution and mounted with DAPI/PI solution. Finally, cells were observed under confocal microscope (Olympus FV1000). Images taken were processed with the support of the flow view version 1.7c software program.

(e) Tubulin polymerization assay: Tubulin polymerization assay was conducted using cytoskeleton kit (BK-004) provided by Lab-pro company. The compounds 4h, 4i, CA-4
(1), nocodazole (Noc), paclitaxel (Pac) were incubated with tubulin (100 µl) that was provided in the kit and the readings were taken for 30 min to 1 h. The O.D at 340 nm was taken and inhibition graph was plotted.

(f) Colchicine binding assay: CytoDYNAMIX Screen™ 15 is designed to detect and measure a compound’s affinity for the colchicine binding site of tubulin. The colchicine binding site is an important modulator of tubulin function, when molecules bind at this site there is inhibition of microtubule polymerization and gives favorable therapeutic index. The competitive colchicine-binding assay is based on a scintillation proximity assay (SPA) technology using bovine brain tubulin, which has been modified so that random surface lysines contain a covalently linked, long-chain biotin derivative (CytoDYNAMIX screen 15, Cytoskeleton, Tebu-Bio). SPA technology requires a close association between a solid phase scintillant (the beads) and the radio-ligand for a signal to be emitted and subsequently detected. Here 10 µL of 20X compound, 10 µL of tritiated colchicne (70-80 Ci/mmol) and 180 µL tubulin beads were used in each well. The entire mixture was incubated for 1hr. Then scintillation readings were taken.

(g) Protein Extraction and Western Blot Analysis: A549 human lung cancer cells were seeded in 60 mm dish and were allowed to grow to attain 80% confluency for 24 h, 2 µM concentration of 2, CA-4 (1), 4h and 4i compounds were added to the culture media, and the cells were incubated with compounds for 24 h. After 24 h total cell lysates from cultured A549 cells were obtained by lysing the cells in ice-cold RIPA buffer (1XPBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) and containing 100 μg/mL PMSF, 5 μg/mL Aprotinin, 5 μg/mL leupeptin, 5 μg/mL pepstatin and 100 μg/mL NaF. After centrifugation at 12,000 rpm for 10 min, the protein in supernatant was quantified by Bradford method (BIO-RAD) using Multimode varioskan instrument (Thermo-Fischer Scientifics). 50 μg of protein per lane was applied in 12 % SDS-polyacrylamide gel. After electrophoresis, the protein was transferred to polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences). The membrane was blocked at room temperature for 2 h in TBS + 0.1% Tween20 (TBST) containing 5 % blocking powder (Santacruz). The membrane was washed with TBST for 5 min, and primary antibody was added and incubated at 4 °C overnight (O/N). Active caspase-3 and β-actin was purchased from
Imagenex Company. Membranes were washed with TBST three times for 15 min and the blots were visualized with chemiluminescence reagent (Thermo Fischer Scientifics Ltd.). The X-ray films were developed with developer and fixed with fixer solution (from Kodak Company).

(h) Docking simulations: Tubulin with colchicine (PDB code: 3E22) was selected as the receptor for docking simulation. After removing the ligand and solvent molecules, hydrogen atoms and Kollman charges were added to each protein atom. Coordinates of each compound were generated using Chemdraw11 followed by MM2 energy minimization. Docking was carried out by AutoDock4 in colchicines binding pocket. Grid map in Autodock that defines the interaction of protein and ligands in binding pocket was defined. The grid map was used with 60 points in each x, y, and z direction, equally spaced at 0.375 Å. Docking was performed using the Lamarckian genetic algorithm. Each docking experiment was performed 100 times, yielding 100 docked conformations. Parameters used for the docking were as follows: population size of 150; random starting position and conformation; maximal mutation of 2 Å in translation and 50 degrees in rotations; elitism of 1; mutation rate of 0.02 and crossover rate of 0.8; and local search rate of 0.06. Simulations were performed with a maximum of 1.5 million energy evaluations and a maximum of 50000 generations. Final docked conformations were clustered using a tolerance of 1.0 Å root mean square deviation. The best model was picked based on the best stabilization energy.
Figure 6: Effect of compound 4h and 4i on γ-tubulin. A549 cells were treated with compounds CA-4 (1), nocodazole (Noc), paclitaxel (Pac), 4h and 4i at 2 µM concentrations for 24 h. γ-Tubulin staining was clearly observed by green colour in control cells and was found to be disrupted and decreased in CA-4 (1), nocodazole, 4h and 4i treated cells. Paclitaxel works as microtubule stabilizer.
Spectral Data and Procedure of Compounds

4-(Benzyloxy)-3-nitrobenzaldehyde (8)

To a stirred solution of 4-hydroxy-3-nitrobenzaldehyde (7, 2.00 g, 12 mmol) in dry acetone (20 mL) was added, anhydrous K$_2$CO$_3$ (3.33 g, 24 mmol), benzyl bromide (186 mg, 2 mmol) and the reaction mixture was stirred at reflux temperature for 12h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, the reaction mixture was cooled to room temperature, filtered and acetone was removed under reduced pressure and the reaction mixture was dissolved in ethyl acetate (30 mL), washed with water (1 x 10 mL) as well as brine (1 x 10 mL) and was dried over anhydrous Na$_2$SO$_4$. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (1:9) as eluent to afford compound 8 as a pale yellow solid (2.82 g, 91%); mp 91–93 °C; $^1$H NMR (CDCl$_3$, 300 MHz): 5.30 (s, 2H, –C$_2$H$_5$–Ph), 7.15 (d, 1H, $J$ = 9.0 Hz, ArH), 7.30–7.39 (m, 5H, ArH), 7.82 (dd, 1H, $J$ = 2.8, 9.0 Hz, ArH), 8.21 (d, 1H, $J$ = 2.8 Hz, ArH), 10.76 (s, 1H, –CHO); MS (ESI): $m/z$ 258 [M+1]$^+$. 

4-(Benzyloxy)-3-nitrobenzoic acid (9)

To a stirred solution of 8 (2.82 g, 11 mmol) in N,N-dimethylformamide (10 mL) was added, oxone (2.43 g, 16 mmol) and stirred for overnight. After completion of the reaction as indicated by TLC, the reaction mixture was poured into ice cold water and extracted with ethyl acetate (3 x 20 mL). The combined extract was washed with water (1 x 20 mL) as well as brine (1 x 20 mL) and was dried over anhydrous Na$_2$SO$_4$. The organic layer was concentrated under reduced pressure to get crude acid product 9 as a pale yellow solid (2.86 g, 95%), which was used directly in the next step without purification; MS (ESI): $m/z$ 274 [M+1]$^+$. 

N1-(3,4,5-Trimethoxyphenyl)-4-(benzyloxy)-3-nitrobenz-amide (10)

To a stirred solution of 9 (2.73 g, 10 mmol) in benzene (35 mL) was treated with thionyl chloride (5.95 g, 50 mmol), catalytic amount of N,N-dimethylformamide (2-3 drops) under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 4h. After completion of the reaction, benzene was removed under reduced pressure to
obtain acid chloride as yellow solid. Owing to potential stability problems, this acid chloride was taken directly to the next step. Triethylamine (2.02 g, 20 mmol) was added drop wise to a stirred solution of 3,4,5-trimethoxyaniline (1.83 g, 10 mmol) in dry tetrahydrofuran (15 mL) at 0 °C; acid chloride was dissolved in dry tetrahydrofuran (10 mL) was then slowly added over 10 min at the same temperature. The reaction was brought to room temperature and stirred for another 2h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, tetrahydrofuran was removed under reduced pressure. The resulting crude mass was diluted with water and extracted with chloroform (2 x 30 mL). The combined extract was washed with saturated NaHCO₃ (1 x 20 mL), water (1 x 20 mL) as well as brine (1 x 20 mL) and was dried over anhydrous Na₂SO₄. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (5:5) as eluent to afford compound 10 as a pale yellow solid (3.72 g, 85%); mp 195–197 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.83 (s, 6H, 2×–OC₃H₃), 3.87 (s, 3H, –OC₃H₃), 5.32 (s, 2H, –CH₂–Ph), 6.94 (s, 2H, ArH), 7.23 (d, 1H, J = 8.9 Hz, ArH), 7.34–7.37 (m, 1H, ArH), 7.41 (d, 2H, J = 7.6 Hz, ArH), 7.45 (d, 2H, J = 6.3 Hz, ArH), 7.78 (s, 1H, –CONH), 8.08 (dd, 1H, J = 2.5, 8.9 Hz, ArH), 8.35 (d, 1H, J = 2.5 Hz, ArH); MS (ESI): m/z 439 [M+1]⁺.

N1-(3,4,5-Trimethoxyphenyl)-4-(benzyloxy)-3-nitro-1-benzene-carbothioamide (11)

A mixture of 10 (4.38 g, 10 mmol) and Lawesson’s reagent (2.42 g, 6 mmol) were taken in dry toluene (25 mL) and refluxed under nitrogen atmosphere for 4h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, the reaction mixture was cooled to room temperature, toluene was removed under reduced pressure and the yellow syrup was dissolved in chloroform (1 x 30 mL), washed with water (1 x 15 mL) and brine (1 x 15 mL) and was dried over Na₂SO₄. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate-hexane (5:5) as eluent to afford compound 11 as a yellow solid (3.45 g, 76%); mp 147–149 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.82 (s, 6H, 2×–OCH₃), 3.86 (s, 3H, –OCH₃), 5.30 (s, 2H, –CH₂–Ph), 6.92 (s, 2H, ArH), 7.22 (d, 1H, J = 8.4 Hz, ArH), 7.34-7.36 (m, 1H, ArH), 7.39 (d, 2H, J = 6.8
Hz, ArH), 7.42 (d, 2H, J = 6.1 Hz, ArH), 7.81 (s, 1H, –CSNH), 8.07 (dd, 1H, J = 2.6, 8.4 Hz, ArH), 8.34 (d, 1H, J = 2.6 Hz, ArH); MS (ESI): m/z 455 [M+1].

N"1-(3,4,5-Trimethoxyphenyl)-4-(benzyloxy)-3-nitro-1-benzene-carboximido hydrazide (12)

To a stirred solution of 11 (3.63 g, 8 mmol) in 20 mL of EtOH-DCM (1:1), NH₂NH₂·H₂O (1.24 g, 24 mmol) was added and stirred at room temperature for 6−8h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, the solvent and excess hydrazine was evaporated under vacuum, and the syrup was dissolved in chloroform (30 mL). The organic layer was washed with water (1 x 10 mL) and brine (1 x 10 mL), and was dried over Na₂SO₄. The organic layer was concentrated under reduced pressure to afford 12 as a yellow solid (2.75 g, 76%), which was directly used in the next step without purification; MS (ESI): m/z 453 [M+1].

5-[4-(Benzyloxy)-3-nitrophenyl]-1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazole (13)

To a stirred solution of 12 (2.72 g, 6 mmol) in 5 ml of AcOH, NaN₂O₂ (0.83 g, 12 mmol) was added portion wise slowly at room temperature for 10 min, and the reaction mixture was stirred for 4−6h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, neutralize with saturated NaHCO₃ solution, and extracted with ethyl acetate (3 x 15 mL). The combined extract was washed with water (1 x 10 mL) as well as brine (1 x 10 mL) and was dried over anhydrous Na₂SO₄. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate-hexane (4:6) as eluent to afford compound 13 as a pale yellow solid (1.83 g, 74%); mp 158−167 °C; 1H NMR (CDCl₃, 300 MHz): δ 3.81 (s, 6H, 2×–OC₃H₃), 3.90 (s, 3H, –OC₃H₃), 5.28 (s, 2H, –CH₂–Ph), 6.61 (s, 2H, ArH), 7.16 (d, 1H, J = 8.8 Hz, ArH), 7.31-7.39 (m, 5H, ArH), 7.80 (dd, 1H, J = 2.2, 8.8 Hz, ArH), 8.19 (d, 1H, J = 2.2 Hz, ArH); MS (ESI): m/z 464 [M+1].

2-Nitro-4-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]-phenol (14)
To a stirred solution of 13 (2.31 g, 5 mmol) in dry dichloromethane (20 mL), TiCl₄ (1.32 g, 7 mmol) was added at 0 °C under nitrogen atmosphere and the reaction mixture was stirred for 30–45 min. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, saturated NaHCO₃ was added to quench the excess TiCl₄ and filtered through celite bed. The organic layer was separated, washed with water (1 x 10 mL), as well as brine (1 x 10 mL) and was dried over anhydrous Na₂SO₄. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate-hexane (5:5) as eluent to afford compound 14 as a pale yellow solid (1.67 g, 90%); mp 145–147 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.83 (s, 6H, 2×–OC₃H₃), 3.92 (s, 3H, –OC₃H₃), 6.60 (s, 2H, ArH), 7.23 (d, 1H, J = 9.0 Hz, ArH), 7.89 (dd, 1H, J = 2.2, 8.3 Hz, ArH), 8.42 (d, 1H, J = 2.2 Hz, ArH); MS (ESI): m/z 374 [M+1]+.

5-[4-(Allyloxy)-3-nitrophenyl]-1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazole (15)
To a stirred solution of compound 14 (1.49 g, 4 mmol) and anhydrous K₂CO₃ (1.39 g, 10 mmol) in dry N,N-dimethylformamide (5 mL), allyl bromide (0.65 g, 5 mmol) was added at 0 °C and the reaction mixture was stirred for 10–12h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, the reaction mixture was poured into ice cold water and extracted with ethyl acetate (3 x 15 mL). The combined extract was washed with water (1 x 10 mL) as well as brine (1 x 10 mL) and was dried over anhydrous Na₂SO₄. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate-hexane (4:6) as eluent to afford compound 15 as a pale yellow solid (1.45 g, 88%); mp 115–117 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.84 (s, 6H), 3.91 (s, 3H), 4.72 (d, 2H, J = 4.5 Hz), 5.35 (d, 1H, J = 10.5 Hz), 5.49 (d, 1H, J = 17.3 Hz), 5.95–6.08 (m, 1H), 6.63 (s, 2H), 7.09 (d, 1H, J = 9.0 Hz), 7.78 (dd, 1H, J = 2.2, 9.0 Hz), 8.05 (d, 1H, J = 2.2 Hz); MS (ESI): m/z 414 [M+1]+.

3-(4-(tert-Butyldimethylsilyloxy)-3-methoxyphenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]-phenoxy-methyl)-4,5-dihydroisoxazole (16a)
To a stirred solution of oxime $6a$ (532 mg, 2 mmol) in dichloromethane (10 mL), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) was added drop wise (over a period of 1h) at 0 °C, later compound $15$ (826 mg, 2 mmol) and triethylamine (20 mg, 0.2 mmol) were dissolved in dichloromethane (5 mL) and added slowly at the same temperature. The reaction was brought to room temperature and stirred for 24h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, water was added to the reaction mixture and the aqueous layer was extracted with dichloromethane (2 x 10 mL). The combined extract was washed with water (1 x 5 mL) as well as brine (1 x 5 mL) and was dried over anhydrous Na$_2$SO$_4$. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate-hexane (6:4) as eluent to afford compound $16a$ as pale yellow solid (996 mg, 72%); mp 196–198 °C; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 0.24 (s, 6H, –S(CH$_3$)$_2$), 0.95 (s, 9H, –C(CH$_3$)$_3$), 3.41–3.54 (m, 2H, isoxazoline–CH$_2$), 3.82 (s, 6H, 2×–OCH$_3$), 3.83 (s, 3H, –OCH$_3$), 3.91 (s, 3H, –OCH$_3$), 4.23–4.26 (m, 1H, –OC$_2$H$_2$), 4.33–4.36 (m, 1H, –OC$_2$H$_2$), 5.05–5.12 (m, 1H, isoxazoline–CH), 6.61 (s, 2H, ArH), 6.82 (d, 1H, $J$ = 8.3 Hz, ArH), 7.19 (d, 1H, $J$ = 8.7 Hz, ArH), 7.31 (d, 1H, $J$ = 1.9 Hz, ArH), 7.83 (d, 1H, $J$ = 8.7 Hz, ArH); MS (ESI): $m/z$ 693 [M+1]$^+$.  

3-(3-(tert-Butyldimethylsilyloxy)-4-methoxyphenyl)-5-(2-nitro-1H,1,2,3,4-tetraazol-5-yl-phenoxy-methyl)-4,5-dihydroisoxazole ($16b$)  

The compound $16b$ was prepared by following the method described for compound $16a$, by employing $15$ (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime $6b$ (532 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound $16b$ as a yellow solid (982 mg, 71%); mp 185–187 °C; $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 0.24 (s, 6H, –S(CH$_3$)$_2$), 0.95 (s, 9H, –C(CH$_3$)$_3$), 3.44 (d, 1H, $J$ = 5.28 Hz, isoxazoline–CH$_2$), 3.47 (d, 1H, $J$ = 7.5 Hz, isoxazoline–CH$_2$), 3.82 (s, 6H, 2×–OCH$_3$), 3.83 (s, 3H, –OCH$_3$), 3.91 (s, 3H, –OCH$_3$), 4.22–4.28 (m, 1H, –OCH$_2$–), 4.31–4.36 (m, 1H, isoxazoline–CH), 6.60 (s, 2H, ArH), 6.82 (d, 1H, $J$ = 8.3 Hz, ArH), 7.12 (dd, 1H, $J$ = 2.2, 8.3 Hz, ArH), 7.21 (d, 2H, $J$ = 2.2 Hz, ArH), 7.83
(dd, 1H, J = 2.2, 8.3 Hz, ArH), 8.11 ppm (d, 1H, J = 2.2 Hz, ArH); MS (ESI): m/z 693 [M+1]⁺.

3-(3,4-Dimethoxyphenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxy-phenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxy)methyl)-4,5-dihydro-isoxazole (16c)

The compound 16c was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6c (362 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16c as a yellow solid (805 mg, 68%); mp 165–167 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.48 (d, 1H, J = 1.5 Hz, isoxazoline–CH₂), 3.50 (d, 1H, J = 3.7 Hz, isoxazoline–CH₂), 3.82 (s, 6H, 2×–OCH₃), 3.90 (s, 6H, 2×–OCH₃), 3.91 (s, 3H, –OCH₃), 4.24–4.29 (m, 1H, –OCH₂–), 4.32–4.37 (m, 1H, –OCH₂–), 5.04–5.13 (m, 1H, isoxazoline–CH), 6.60 (s, 2H, ArH), 6.83 (d, 1H, J = 8.3 Hz, ArH), 7.03 (dd, 1H, J = 2.2, 8.3 Hz, ArH), 7.19 (d, 1H, J = 9.0 Hz, ArH), 7.33 (d, 1H, J = 2.2 Hz, ArH), 7.83 (dd, 1H, J = 2.2, 9.0 Hz, ArH), 8.10 (d, 1H, J = 2.2 Hz, ArH); MS (ESI): m/z 593 [M+1]⁺.

3-(2,5-Dimethoxyphenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxy-phenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxy)methyl)-4,5-dihydro-isoxazole (16d)

The compound 16d was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6d (362 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16d as a yellow solid (888 mg, 75%); mp 141–143 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.60 (d, 2H, J = 7.3 Hz, isoxazoline–CH₂), 3.77 (s, 3H, –OCH₃), 3.82 (s, 6H, 2×–OCH₃), 3.91 (s, 3H, –OCH₃), 4.24–4.34 (m, 2H, –OCH₂–), 5.00–5.09 (m, 1H, isoxazoline–CH), 6.60 (s, 2H, ArH), 6.85 (d, 1H, J = 9.0 Hz, ArH), 7.23 (d, 2H, J = 9.4 Hz, ArH), 7.82 (dd, 1H, J = 1.1, 8.8 Hz, ArH), 8.10 (d, 1H, J = 2.0 Hz, ArH); MS (ESI): m/z 593 [M+1]⁺.

5-(2-Nitro-4-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxy)methyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxa-zole (16e)
The compound 16e was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6e (422 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16e as a yellow solid (909 mg, 73%); mp 231–233 °C; 1H NMR (CDCl3, 300 MHz): δ 3.52–3.54 (m, 2H, isoxazoline–CH2), 3.82 (s, 6H, 2×–OCH3), 3.88 (s, 3H, –OC6H3), 3.89 (s, 6H, 2×–OC6H3), 3.93 (s, 3H, –OCH3), 4.30–4.35 (m, 2H, –OC6H2–), 5.14–5.19 (m, 1H, isoxazoline–CH), 6.61 (s, 2H, ArH), 6.90 (s, 2H), 7.19 (d, 1H, J = 9.1 Hz), 7.83 (d, 1H, J = 9.1 Hz), 8.10 (s, 1H); MS (ESI): m/z 623 [M+1]+.

5-(2-Nitro-4-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-3-(2,3,4-trimethoxyphenyl)-4,5-dihydroisoxazole (16f)

The compound 16f was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6f (422 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16f as a yellow solid (920 mg, 74%); mp 124–126 °C; 1H NMR (CDCl3, 300 MHz): δ 3.52–3.54 (m, 2H, isoxazoline–CH2), 3.81 (s, 6H, 2×–OC6H3), 3.88 (s, 3H, –OC6H3), 3.91 (s, 6H, 2×–OCH3), 3.92 (s, 3H, –OCH3), 4.30–4.35 (m, 2H, –OC6H2–), 5.14–5.19 (m, 1H, isoxazoline–CH), 6.61 (s, 2H, ArH), 6.70 (d, 1H, J = 8.9 Hz, ArH), 7.18 (d, 1H, J = 8.9 Hz, ArH), 7.46 (d, 1H, J = 8.9 Hz, ArH), 7.83 (d, 1H, J = 8.9 Hz, ArH), 8.08 (d, 1H, J = 1.8 Hz, ArH); MS (ESI): m/z 623 [M+1]+.

3-(4-Methoxy-3-nitrophenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-4,5-dihydroisoxazole (16g)

The compound 16g was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6g (392 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16g as a yellow solid (860 mg, 71%); mp 171–173 °C; 1H NMR (CDCl3, 300 MHz): δ 3.54 (d, 2H, J = 8.6 Hz, isoxazoline–CH2), 3.82 (s, 6H, 2×–OCH3), 3.91 (s, 3H, –OCH3), 4.01 (s, 3H, –OCH3), 4.28–4.40 (m, 2H, –OC6H2–), 5.10–5.19 (m, 1H,
isoxazoline–C\(\text{H}\), 6.60 (s, 2H, Ar\(\text{H}\)), 7.12 (d, 1H, \(J = 8.8\) Hz, Ar\(\text{H}\)), 7.17 (d, 1H, \(J = 8.8\) Hz, Ar\(\text{H}\)), 7.84 (dd, 1H, \(J = 2.2, 8.8\) Hz, Ar\(\text{H}\)), 7.90 (dd, 1H, \(J = 2.2, 8.8\) Hz, Ar\(\text{H}\)), 8.02 (d, 1H, \(J = 2.0\) Hz, Ar\(\text{H}\)), 8.10 (d, 1H, \(J = 2.0\) Hz, Ar\(\text{H}\)); MS (ESI): \(m/z\) 608 [M+1]+.

3-(2-Fluoro-5-methoxyphenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxy-phenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxy)methyl)-4,5-dihydro-isoxazole (16h)

The compound 16h was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6h (340 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16h as a yellow solid (870 mg, 75%); mp 178–180 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 3.55–3.61 (m, 2H, isoxazoline–C\(\text{H}\)\(_2\)), 3.82 (s, 6H, 2×–OC\(\text{H}\)_3), 3.89 (s, 3H, –OC\(\text{H}\)_3), 3.92 (s, 3H, –OC\(\text{H}\)_3), 4.29 (d, 2H, \(J = 4.8\) Hz, –OC\(\text{H}\)_3), 5.05–5.12 (m, 1H, isoxazoline–C\(\text{H}\)), 6.62 (s, 2H, Ar\(\text{H}\)), 7.06–7.14 (m, 2H, Ar\(\text{H}\)), 7.18 (d, 1H, \(J = 9.0\) Hz, Ar\(\text{H}\)), 7.35–7.39 (m, 1H, Ar\(\text{H}\)), 7.82 (d, 1H, \(J = 8.6\) Hz, Ar\(\text{H}\)), 8.08 (d, 1H, \(J = 2.1\) Hz, Ar\(\text{H}\)); MS (ESI): \(m/z\) 581 [M+1]+.

3-(2-Fluorophenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxy-phenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxy)methyl)-4,5-dihydroisoxazole (16i)

The compound 16i was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6i (278 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16i as a yellow solid (803 mg, 73%); mp 181–183 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 3.56–3.63 (m, 2H, isoxazoline–C\(\text{H}\)\(_2\)), 3.82 (s, 6H, 2×–OC\(\text{H}\)_3), 3.90 (s, 3H, –OC\(\text{H}\)_2–), 4.30 (d, 2H, \(J = 4.5\) Hz, –OC\(\text{H}\)_2–), 5.05–5.14 (m, 1H, isoxazoline–C\(\text{H}\)), 6.61 (s, 2H, Ar\(\text{H}\)), 7.06–7.14 (m, 1H, Ar\(\text{H}\)), 7.17 (d, 2H, \(J = 9.0\) Hz, Ar\(\text{H}\)), 7.35–7.42 (m, 1H, Ar\(\text{H}\)), 7.80 (t, 2H, \(J = 7.5\) Hz, Ar\(\text{H}\)), 8.06 (d, 1H, \(J = 2.2\) Hz, Ar\(\text{H}\)); MS (ESI): \(m/z\) 551 [M+1]+.

3-(3-Chlorophenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxy-phenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxy)methyl)-4,5-dihydroisoxazole (16j)

The compound 16j was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6j (278 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16j as a yellow solid (803 mg, 73%); mp 181–183 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 3.56–3.63 (m, 2H, isoxazoline–C\(\text{H}\)\(_2\)), 3.82 (s, 6H, 2×–OC\(\text{H}\)_3), 3.90 (s, 3H, –OC\(\text{H}\)_2–), 4.30 (d, 2H, \(J = 4.5\) Hz, –OC\(\text{H}\)_2–), 5.05–5.14 (m, 1H, isoxazoline–C\(\text{H}\)), 6.61 (s, 2H, Ar\(\text{H}\)), 7.06–7.14 (m, 1H, Ar\(\text{H}\)), 7.17 (d, 2H, \(J = 9.0\) Hz, Ar\(\text{H}\)), 7.35–7.42 (m, 1H, Ar\(\text{H}\)), 7.80 (t, 2H, \(J = 7.5\) Hz, Ar\(\text{H}\)), 8.06 (d, 1H, \(J = 2.2\) Hz, Ar\(\text{H}\)); MS (ESI): \(m/z\) 551 [M+1]+.
The compound 16j was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6j (311 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16j as a yellow solid (792 mg, 70%); mp 185–188 °C; 1H NMR (CDCl₃, 300 MHz): δ 3.48 (t, 2H, J = 4.53 Hz, isoxazoline–CH₂), 3.82 (s, 6H, 2×–OC₃H₃), 3.89 (s, 3H, –OC₃H₃), 4.30 (t, 2H, J = 4.5 Hz, –OC₂H₂–), 5.06–5.15 (m, 1H, isoxazoline–CH), 6.60 (s, 2H, ArH), 7.18 (d, 1H, J = 9.0 Hz, ArH), 7.35 (d, 2H, J = 6.8 Hz, ArH), 7.52 (d, 1H, J = 9.0 Hz, ArH), 7.62 (s, 1H, ArH), 7.81 (dd, 1H, J = 2.2, 9.0 Hz, ArH), 8.04 (d, 1H, J = 2.2 Hz, ArH); MS (ESI): m/z 567 [M+1]+.

5-(2-Nitro-4-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxy)methyl-3-[4-(trifluoromethyl)phenyl]-4,5-dihydro-isoxazole (16k)

The compound 16k was prepared by following the method described for compound 16a, by employing 15 (826 mg, 1 mmol) and triethylamine (20 mg, 0.2 mmol), 13% aqueous solution of NaOCl (1.72 g, 3 mmol) and oxime 6k (378 mg, 2 mmol), the crude product was purified by column chromatography (60% ethyl acetate–hexane) to afford the compound 16k as a yellow solid (852 mg, 71%); mp 165–168 °C; 1H NMR (CDCl₃, 300 MHz): δ 3.40–3.49 (m, 1H, isoxazoline–CH₂), 3.60–3.70 (m, 1H, isoxazoline–CH₂), 3.79 (s, 6H, 2×–OC₃H₃), 3.91 (s, 3H, –OC₃H₃), 4.16 (d, 2H, J = 4.5 Hz, –OC₂H₂–), 5.09–5.18 (m, 1H, isoxazoline–CH), 6.61 (s, 2H, ArH), 6.74 (d, 1H, J = 8.3 Hz, ArH), 6.81 (dd, 1H, J = 1.5, 8.3 Hz, ArH), 7.08 (d, 1H, J = 2.2 Hz, ArH), 7.09–7.17 (m, 1H, ArH), 7.19 (d, 1H, J = 6.8 Hz, ArH), 7.37–7.45 (m, 1H, ArH), 7.84–7.89 (m, 1H, ArH); MS (ESI): m/z 601 [M+1]+.

2-Methoxy-4-(5-(2-nitro-4-(1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl)phenoxy)methyl)-4,5-dihydro-3-isoxazolyl)-phenol (17a)

A solution of silyl ether 17a (760 mg, 1.1 mmol) in dry tetrahydrofuran (10 mL) was treated with tetrabutylammonium fluoride (378 mg, 1.2 mmol), stirring was continued for 20 min, and then ice water (5 mL) was added, followed by diethyl ether. The ethereal layer was washed with water (3 x 10 mL) and dried over anhydrous Na₂SO₄. The organic layer was concentrated under reduced pressure and the crude product was purified by
column chromatography (silica gel; 60-120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 17a as a white solid (580 mg, 91%); mp 189–191 °C; 1H NMR (CDCl₃, 300 MHz): δ 3.43–3.54 (m, 2H, isoxazoline–C–H₂), 3.82 (s, 6H, 2×–OCH₃), 3.84 (s, 3H, –OCH₃), 3.92 (s, 3H, –OCH₃), 4.23–4.25 (m, 1H, –OCH₂–), 4.33–4.37 (m, 1H, –OCH₂–), 5.04–5.10 (m, 1H, isoxazoline–CH), 6.62 (s, 2H, ArH), 6.82 (d, 1H, J = 7.8 Hz, ArH), 6.98 (dd, 1H, J = 2.1, 7.9 Hz, ArH), 7.20 (d, 1H, J = 8.8 Hz, ArH), 7.31 (d, 1H, J = 2.1 Hz, ArH), 7.84 (d, 1H, J = 8.9 Hz, ArH); MS (ESI): m/z 579 [M+1]^+.

2-Methoxy-5-((2-nitro-4-(1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl)phenoxy)methyl)-4,5-dihydro-3-isoxazolyl-phenol (17b)

The compound 17b was prepared by following the method described for compound 17a, by employing 16b (760 mg, 1.1 mmol) and tetrabutylammonium fluoride (378 mg, 1.2 mmol), the crude product was purified by column chromatography (silica gel; 60-120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 17b as a white solid (565 mg, 89%); mp 182–184 °C; 1H NMR (CDCl₃, 300 MHz): δ 3.45 (d, 1H, J = 5.3 Hz, isoxazoline–C–H₂), 3.47 (d, 1H, J = 7.5 Hz, isoxazoline–C–H₂), 3.82 (s, 6H, 2×–OCH₃), 3.84 (s, 3H, –OCH₃), 3.91 (s, 3H, –OCH₃), 4.23–4.27 (m, 1H, –OCH₂–), 4.30–4.36 (m, 1H, –OCH₂–), 5.03–5.09 (m, 1H, isoxazoline–CH), 6.61 (s, 2H, ArH), 6.82 (d, 1H, J = 8.3 Hz, ArH), 7.13 (dd, 1H, J = 2.3, 8.5 Hz, ArH), 7.21 (d, 2H, J = 2.3 Hz, ArH), 7.82 (dd, 1H, J = 2.3, 8.5 Hz, ArH), 8.10 (d, 1H, J = 2.3 Hz, ArH); MS (ESI): m/z 579 [M+1]^+.

2-(Allyloxy)-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (18)

To a solution of compound 15 (413 mg, 1 mmol) in acetic acid (5 mL), Zn dust (196 mg, 3 mmol) was added and the reaction mixture was stirred at room temperature for 2–3h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, the reaction mixture was filtered, neutralized with saturated NaHCO₃ and extracted with ethyl acetate (3 x 10 mL). The combined extract was washed with water (1 x 5 mL) as well as brine (1 x 5 mL) and was dried over anhydrous Na₂SO₄. The organic layer was concentrated under reduced pressure the crude product 18 (325 mg, 85%), which was directly used in the next step without purification; MS (ESI): m/z 414 [M+1]^+. 

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4-(2-Amino-4-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-4,5-dihydro-3-isoxazolyl]-2-methoxyphenol (4a)

To a solution of compound 17a (578 mg, 1 mmol) in acetic acid (5 mL), Zn dust (196 mg, 3 mmol) was added and the reaction mixture was stirred at room temperature for 2–3h. The progress of the reaction was monitored by TLC for completion. After completion of the reaction, the reaction mixture was filtered, neutralized with saturated NaHCO₃ and extracted with ethyl acetate (3 x 10 mL). The combined extract was washed with water (1 x 5 mL) as well as brine (1 x 5 mL) and was dried over anhydrous Na₂SO₄. The organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4a as a white solid (450 mg, 82%); mp 175–178 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.32–3.43 (m, 1H, isoxazoline–CH₂), 3.55–3.63 (m, 1H, isoxazoline–CH₂–), 3.81 (s, 6H, 2×–OC₃H₃), 3.89 (s, 3H, –OC₃H₃), 3.91 (s, 3H, –OC₃H₃), 4.21 (d, 2H, J = 3.2 Hz, –OC₃H₂–), 5.05–5.13 (m, 1H, isoxazoline–CH), 6.88 (t, 2H, J = 8.1 Hz, ArH), 7.01 (s, 2H, ArH), 7.04 (d, 1H, J = 8.5 Hz, ArH), 7.14 (d, 1H, J = 8.3 Hz, ArH), 7.24 (s, 1H, ArH), 7.30 (s, 1H, ArH); ¹³C NMR (CDCl₃+[D₆] DMSO, 75 MHz): δ 38.1, 55.8, 55.8, 59.8, 68.1, 78.5, 104.1, 116.3, 116.8, 119.0, 120.8, 121.1, 125.2, 128.6, 129.1, 135.0, 137.2, 138.6, 149.7, 153.3, 153.5, 153.9, 154.4, 157.9 ppm; IR (KBr) (νmax/cm⁻¹): 1133, 1231, 1294, 1433, 1460, 1493, 1516, 1600, 3363, 3419, 3465; MS (ESI): m/z 549 [M+1]⁺; HRMS (ESI m/z) for C₂₇H₂₈N₆O₇ calcd 549.2248, found 549.2253 [M+1]+.

5-[5-(2-Amino-4-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-4,5-dihydro-3-isoxazolyl]-2-methoxyphenol (4b)

The compound 4b was prepared by following the method described for compound 4a, by employing 17b (578 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4b as a white solid (438 mg, 80%); mp 165–168 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.26–3.34 (m, 1H, isoxazoline–CH₂), 3.46–3.55 (m, 1H, isoxazoline–CH₂), 3.79 (s, 6H, 2×–OCH₃), 3.84 (s, 3H, –OCH₃), 3.91 (s, 3H, –OCH₃), 4.15 (d, 2H, J = 4.5 Hz, –OCH₂–), 5.05–5.13 (m, 1H, isoxazoline–CH), 6.60 (s,
2H, ArH), 6.74 (d, 1H, J = 8.5 Hz, ArH), 6.80 (dd, 1H, J = 1.9, 8.5 Hz, ArH), 6.84 (d, 1H, J = 8.3 Hz, ArH), 7.07 (d, 1H, J = 1.9 Hz, ArH), 7.15 (dd, 1H, J = 1.9, 8.3 Hz, ArH), 7.27 (s, 1H, ArH); 13C NMR (CDCl3+[D6] DMSO, 75 MHz): δ 37.8, 54.1, 56.2, 60.1, 68.4, 77.8, 104.2, 116.1, 116.9, 117.1, 119.2, 121.4, 124.9, 126.2, 131.0, 134.5, 136.6, 137.9, 149.9, 152.8, 153.0, 153.2, 154.1, 157.5 ppm; IR (KBr) (ν max/cm–1): 1131, 1230, 1296, 1346, 1417, 1457, 1495, 1514, 1596, 1624, 3361, 3465, 3546; MS (ESI): m/z 549 [M+1]+; HRMS (ESI m/z) for C27H28N6O7 calcd 549.2248, found 549.2257 [M+1]+.

2-[3-(3,4-Dimethoxyphenyl)-4,5-dihydro-5-isoxazolyl]-methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (4c)

The compound 4c was prepared by following the method described for compound 4a, by employing 16c (592 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4c as a white solid (477 mg, 85%); mp 155–157 ºC; 1H NMR (CDCl3, 300 MHz): δ 3.36 (m, 1H, isoxazoline–CH2), 3.56 (m, 1H, isoxazoline–CH2), 3.77 (s, 6H, 2×–OCH3), 3.78 (s, 3H, –OCH3), 3.83 (s, 3H, –OCH3), 3.84 (s, 3H, –OCH3), 4.16 (d, 2H, J = 4.3 Hz, –OCH2–), 5.03–5.14 (m, 1H, isoxazoline–CH), 6.65 (s, 2H, ArH), 7.00 (d, 1H, J = 7.5 Hz, ArH), 7.05 (d, 1H, J = 8.6 Hz, ArH), 7.11 (dd, 1H, J = 1.5, 9.0 Hz, ArH), 7.21 (d, 1H, J = 8.5 Hz, ArH), 7.32 (d, 1H, J = 8.5 Hz, ArH), 7.44 (d, 1H, J = 1.5 Hz, ArH); 13C NMR (CDCl3, 75 MHz): δ 37.9, 55.1, 55.6, 55.8, 60.4, 69.0, 78.1, 104.5, 114.3, 114.9, 115.8, 119.2, 120.9, 124.5, 126.6, 130.8, 133.9, 137.4, 138.4, 149.9, 152.6, 152.9, 153.0, 153.3, 157.8 ppm; IR (KBr) (ν max/cm–1): 1131, 1232, 1340, 1420, 1458, 1490, 1515, 1598, 3372, 3469; MS (ESI): m/z 563 [M+1]+; HRMS (ESI m/z) for C28H31N6O7 calcd 563.2254, found 563.2251 [M+1]+.

2-[3-(2,5-Dimethoxyphenyl)-4,5-dihydro-5-isoxazolyl]-methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (4d)

The compound 4d was prepared by following the method described for compound 4a, by employing 16d (592 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4d as a white solid (455 mg, 81%); mp 120–122 ºC; 1H NMR (CDCl3, 300 MHz): δ 3.43–3.49 (m, 1H, isoxazoline–CH2), 3.60–3.66
(m, 1H, isoxazoline–CH₂), 3.78 (s, 3H, –OCH₃), 3.79 (s, 6H, 2×–OCH₃), 3.81 (s, 3H, –OCH₃), 3.91 (s, 3H, –OCH₃), 4.13 (d, 2H, J = 4.5 Hz, –OCH₂–), 5.04–5.12 (m, 1H, isoxazoline–CH), 6.61 (s, 2H, ArH), 6.72 (d, 1H, J = 8.3 Hz, ArH), 6.80 (dd, 1H, J = 1.5, 8.3 Hz, ArH), 6.88 (d, 1H, J = 9.0 Hz, ArH), 6.93 (dd, 1H, J = 3.0, 9.0 Hz, ArH), 7.06 (d, 1H, J = 2.2 Hz, ArH), 7.30 (d, 1H, J = 3.0 Hz, ArH); ¹³C NMR (CDCl₃, 75 MHz): δ 37.6, 54.7, 55.4, 56.1, 59.8, 67.8, 78.0, 104.6, 116.8, 118.3, 118.9, 119.6, 121.3, 121.6, 122.1, 123.9, 134.2, 136.8, 138.3, 150.8, 152.3, 153.1, 153.0, 153.6, 157.6 ppm; IR (KBr) (νmax/cm⁻¹): 1127, 1230, 1294, 1340, 1421, 1461, 1498, 1602, 3365, 3461; MS (ESI): m/z 563 [M+1]+; HRMS (ESI m/z) for C₂₈H₃₁N₆O₇ calcd 563.2254, found 563.2251 [M+1]+.

2-[(3-(3,4,5-Trimethoxyphenyl)-4,5-dihydro-5-isoxazolyl)-methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (4e)

The compound 4e was prepared by following the method described for compound 4a, by employing 16e (622 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4e as a white solid (473 mg, 80%); mp 202–204 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.33–3.40 (m, 1H, isoxazoline–CH₂), 3.50–3.59 (m, 1H, isoxazoline–CH₂), 3.79 (s, 6H, 2×–OCH₃), 3.88 (s, 9H, 3×–OCH₃), 3.91 (s, 3H, –OCH₃), 4.16 (d, 2H, J = 4.5 Hz, –OCH₂–), 5.10–5.19 (m, 1H, isoxazoline–CH), 6.61 (s, 2H, ArH), 6.76 (d, 1H, J = 8.3 Hz, ArH), 6.81 (dd, 1H, J = 1.5, 8.3 Hz, ArH), 6.91 (s, 2H, ArH), 7.08 (d, 1H, J = 2.2 Hz, ArH); ¹³C NMR (CDCl₃, 75 MHz): δ 37.5, 56.3, 56.6, 60.1, 60.4, 67.5, 77.8, 104.1, 109.8, 116.8, 118.7, 120.6, 124.1, 131.3, 133.7, 136.5, 139.3, 150.1, 152.1, 152.8, 153.7, 153.9, 157.6 ppm; MS (ESI): m/z 593 [M+1]+; HRMS (ESI m/z) for C₂₉H₃₃N₆O₈ calcd 593.2359, found 593.2375 [M+1]+.

2-[(3-(2,3,4-Trimethoxyphenyl)-4,5-dihydro-5-isoxazolyl)-methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (4f)

The compound 4f was prepared by following the method described for compound 4a, by employing 16f (622 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4f as a white solid (503 mg, 85%); mp 85–87 °C; ¹H NMR (CDCl₃, 300 MHz): δ 3.37–3.45 (m, 1H, isoxazoline–CH₂), 3.57–3.66 (m,
1H, isoxazoline–CH2), 3.79 (s, 6H, 2×–OCH3), 3.86 (s, 3H, –OCH3), 3.90 (s, 6H, 2×–OCH3), 3.91 (s, 3H, –OCH3), 4.15 (d, 1H, J = 4.3 Hz, –OCH2–), 5.05–5.09 (m, 1H, isoxazoline–CH), 6.60 (s, 2H, ArH), 6.68 (d, 1H, J = 8.8 Hz, ArH), 6.72 (d, 1H, J = 8.5 Hz, ArH), 6.80 (dd, 1H, J = 2.0, 8.5 Hz, ArH), 7.06 (d, 1H, J = 1.8 Hz, ArH), 7.44 (d, 1H, J = 8.6 Hz, ArH); 13C NMR (CDCl3, 75 MHz): δ 37.8, 55.7, 55.9, 57.3, 58.7, 59.8, 68.1, 78.2, 104.3, 116.1, 117.5, 119.2, 120.6, 121.2, 124.5, 126.8, 134.2, 137.3, 138.6, 149.5, 152.1, 152.3, 152.9, 153.1, 153.5, 157.2 ppm; IR (KBr) (νmax/cm−1): 1084, 1127, 1232, 1357, 1415, 1461, 1500, 1599, 3372, 3457; MS (ESI): m/z 593 [M+1]+; HRMS (ESI m/z) for C29H33N6O8 calcd 593.2359, found 593.2375 [M+1]+.

2-[3-(3-Amino-4-methoxyphenyl)-4,5-dihydro-5-isoxazol-yl]methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (4g)

The compound 4g was prepared by following the method described for compound 4a, by employing 16g (607 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4g as a white solid (448 mg, 82%); mp 141–143 °C; 1H NMR (CDCl3, 300 MHz): δ 3.30–3.39 (m, 1H, isoxazoline–CH2), 3.46–3.56 (m, 1H, isoxazoline–CH2), 3.79 (s, 6H, 2×–OCH3), 3.88 (s, 3H, –OCH3), 3.91 (s, 3H, –OCH3), 4.14 (d, 2H, J = 4.5 Hz, –OCH2–), 5.02–5.13 (m, 1H, isoxazoline–CH), 6.60 (s, 2H, ArH), 6.75 (d, 1H, J = 2.2 Hz, ArH), 6.68–6.80 (m, 2H, ArH), 6.93–6.97 (m, 1H, ArH), 7.06 (d, 1H, J = 2.2 Hz, ArH), 7.14 (dd, 1H, J = 1.5, 6.0 Hz, ArH); 13C NMR (CDCl3, 75 MHz): δ 37.5, 53.9, 56.1, 60.3, 67.5, 76.8, 104.9, 116.7, 118.4, 118.7, 120.6, 124.0, 126.8, 127.2, 131.2, 134.6, 136.7, 139.5, 142.3, 150.2, 152.6, 152.8, 153.4, 157.5 ppm; IR (KBr) (νmax/cm−1): 1084, 1127, 1232, 1357, 1415, 1461, 1420, 1500, 1599, 3372, 3457; MS (ESI): m/z 593 [M+1]+; HRMS (ESI m/z) for C29H33N6O8 calcd 593.2359, found 593.2375 [M+1]+.

2-[3-(2-Fluoro-5-methoxyphenyl)-4,5-dihydro-5-isoxazol-yl]methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (4h)

The compound 4h was prepared by following the method described for compound 4a, by employing 16h (580 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–
hexane (6:4) as eluent to afford compound 4h as a white solid (421 mg, 79%); mp 164–166 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta 3.41–3.49 \text{(m, 1H, isoxazoline–CH}_2\)) 3.60–3.67 (m, 1H, isoxazoline–CH\(_2\)), 3.81 (m, 6H, 2×–OCH\(_3\)), 3.90 (s, 3H, –OCH\(_3\)), 3.92 (s, 3H, –OCH\(_3\)), 4.15 (d, 2H, \(J = 4.1 \text{ Hz, –OCH}_2\)), 5.09–5.18 (m, 1H, isoxazoline–CH), 6.61 (s, 2H, ArH), 6.75 (d, 1H, \(J = 8.0 \text{ Hz, ArH}\)), 6.80 (dd, 1H, \(J = 1.8, 8.0 \text{ Hz, ArH}\)), 7.08–7.17 (m, 3H, ArH), 7.19 (d, 1H, \(J = 6.8 \text{ Hz, ArH}\)); \(^{13}\)C NMR (CDCl\(_3\)+[D\(_6\)] DMSO, 75 MHz): \(\delta 37.8, 55.5, 55.6, 60.1, 66.8, 77.7, 104.5, 115.1, 116.2, 116.3, 119.9, 121.2, 121.5, 123.7, 125.2, 133.5, 137.0, 137.1, 149.2, 153.1, 153.5, 157.7, 158.3 ppm; IR (KBr) (\(v_{\text{max}}/\text{cm}^{-1}\)): 1026, 1128, 1218, 1299, 1349, 1417, 1455, 1496, 1599, 1623, 3367, 3467; MS (ESI): \(m/z 551 [M+1]^+\); HRMS (ESI \(m/z\)) for C\(_{27}\)H\(_{28}\)N\(_6\)O\(_6\)F calcd 551.2053, found 521.2038 \([M+1]^+\).

### 2-[3-(2-Fluorophenyl)-4,5-dihydro-5-isoxazolyl]methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (4i)

The compound 4i was prepared by following the method described for compound 4a, by employing 16i (550 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4i as a white solid (421 mg, 81%); mp 171–173 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta 3.40–3.49 \text{(m, 1H, isoxazoline–CH}_2\)) 3.59–3.66 (m, 1H, isoxazoline–CH\(_2\)), 3.79 (m, 6H, 2×–OCH\(_3\)), 3.91 (s, 3H, –OCH\(_3\)), 4.16 (d, 2H, \(J = 4.5 \text{ Hz, –OCH}_2\)), 5.09–5.18 (m, 1H, isoxazoline–CH), 6.60 (s, 2H, ArH), 6.74 (d, 1H, \(J = 8.3 \text{ Hz, ArH}\)), 6.80 (dd, 1H, \(J = 1.5, 8.3 \text{ Hz, ArH}\)), 7.07 (d, 1H, \(J = 2.2 \text{ Hz, ArH}\)), 7.12–7.17 (m, 1H, ArH), 7.19 (d, 1H, \(J = 6.8 \text{ Hz, ArH}\)), 7.37–7.45 (m, 1H, ArH), 7.83–7.89 (m, 1H, ArH); \(^{13}\)C NMR (CDCl\(_3\)+[D\(_6\)] DMSO, 75 MHz): \(\delta 38.0, 56.6, 60.5, 68.7, 78.3, 104.7, 116.3, 116.5, 117.4, 119.6, 121.1, 124.8, 124.9, 129.1, 132.3, 134.7, 137.1, 138.3, 150.2, 153.3, 153.6, 158.8, 160.8 ppm; IR (KBr) (\(v_{\text{max}}/\text{cm}^{-1}\)): 1001, 1128, 1230, 1295, 1348, 1420, 1458, 1499, 1600, 3355, 3450; MS (ESI): \(m/z 521 [M+1]^+\); HRMS (ESI \(m/z\)) for C\(_{26}\)H\(_{26}\)N\(_6\)O\(_5\)F calcd 521.2053, found 521.2038 \([M+1]^+\).

### 2-[3-(3-Chlorophenyl)-4,5-dihydro-5-isoxazolyl]methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]aniline (4j)

The compound 4j was prepared by following the method described for compound 4a, by employing 16j (566 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product
was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4j as a white solid (444 mg, 83%); mp 189–191 °C; 1H NMR (CDCl₃, 400 MHz): δ 3.32–3.37 (m, 1H, isoxazoline–CH₂), 3.49–3.55 (m, 1H, isoxazoline–CH₂), 3.78 (s, 6H, 2×–OCH₃), 3.90 (s, 3H, –OCH₃), 4.16 (d, 2H, J = 4.2 Hz, –OCH₂–), 5.13–5.18 (m, 1H, isoxazoline–CH₂), 6.60 (s, 2H, ArH), 6.74 (d, 1H, J = 8.3 Hz, ArH), 6.80 (d, 1H, J = 8.3 Hz, ArH), 7.07 (s, 1H, ArH), 7.35 (t, 1H, J = 8.3 Hz, ArH), 7.39 (d, 1H, J = 7.5 Hz, ArH), 7.56 (s, 1H, ArH); 13C NMR (CDCl₃+[D₆] DMSO, 75 MHz): δ 38.3, 56.2, 61.1, 67.4, 78.0, 104.2, 117.8, 120.6, 120.9, 125.2, 128.5, 130.6, 131.1, 133.4, 133.6, 134.9, 136.7, 140.1, 140.8, 150.8, 153.4, 154.1, 158.1 ppm; IR (KBr) (ν max/cm⁻¹): 1130, 1232, 1294, 1343, 1419, 1459, 1499, 1600, 3365, 3459; MS (ESI): m/z 537 [M+1]⁺; HRMS (ESI m/z) for C₂₆H₂₆N₆O₅Cl calcd 537.1653, found 537.1650 [M+1]⁺.

2-(3-[4-(Trifluoromethyl)phenyl]-4,5-dihydro-5-isoxazolyl-meth-oxy)-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]-aniline (4k)

The compound 4k was prepared by following the method described for compound 4a, by employing 16k (600 mg, 1 mmol) and Zn dust (196 mg, 3 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4k as a white solid (478 mg, 84%); mp 210–212 °C; 1H NMR (CDCl₃, 300 MHz): δ 3.41–3.46 (m, 1H, isoxazoline–CH₂), 3.59–3.64 (m, 1H, isoxazoline–CH₂), 3.78 (s, 6H, 2×–OCH₃), 3.85 (s, 3H, –OCH₃), 4.21 (d, 2H, J = 3.9 Hz, –OCH₂–), 5.15–5.24 (m, 1H, isoxazoline–CH₂), 6.69 (s, 2H, ArH), 6.72 (d, 1H, J = 8.0 Hz, ArH), 6.81 (d, 1H, J = 8.3 Hz, ArH), 7.09 (s, 1H, ArH), 7.71 (d, 2H, J = 7.5 Hz, ArH), 7.85 (d, 2H, J = 6.9 Hz, ArH); 13C NMR (CDCl₃+[D₆] DMSO, 75 MHz): δ 38.7, 55.9, 59.7, 68.1, 77.4, 104.7, 115.9, 119.9, 121.6, 123.9, 126.8, 131.6, 133.2, 134.9, 135.6, 137.7, 138.1, 139.9, 149.7, 153.0, 153.8, 157.5 ppm; IR (KBr) (ν max/cm⁻¹): 1129, 1228, 1295, 1326, 1417, 1459, 1500, 1600, 3367, 3465; MS (ESI): m/z 571 [M+1]⁺; HRMS (ESI m/z) for C₂₇H₂₆N₆O₅F₃ calcd 571.1916, found 571.1921 [M+1]⁺.

2-[3-(4-Methoxy-3-nitrophenyl)-4,5-dihydro-5-isoxazolyl-meth-oxy)-5-[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3,4-tetraazol-5-yl]-aniline (4l)

The compound 4l was prepared by following the method described for compound 16a, by employing 17 (383 mg, 1 mmol) and Et₃N (20 mg, 0.2 mmol), 13% aqueous solution of
NaOCl (1.72 g, 3 mmol) and oxime 6g (392 mg, 2 mmol), and the crude product was purified by column chromatography (silica gel; 60–120 mesh) using ethyl acetate–hexane (6:4) as eluent to afford compound 4l as a pale yellow solid (237 mg, 41%); mp 159–161 ºC; 1H NMR (CDCl₃, 300 MHz): δ 3.31–3.41 (m, 1H, isoxazoline–CH₂), 3.49–3.62 (m, 1H, isoxazoline–CH₂), 3.71 (s, 3H, –OCH₃), 3.79 (s, 3H, –OCH₃), 3.91 (s, 3H, –OCH₃), 4.02 (s, 3H, –OCH₃), 4.19 (d, 1H, J = 4.3 Hz, –OCH₂), 4.24 (d, 1H, J = 4.3 Hz, –OCH₂), 5.15–5.24 (m, 1H, isoxazoline–CH), 6.57 (s, 1H, ArH), 6.61 (s, 1H, ArH), 6.82 (s, 1H, ArH), 7.17 (dd, 2H, J = 2.4, 8.8 Hz, ArH), 7.34 (d, 1H, J = 9.0 Hz, ArH), 7.98 (dd, 1H, J = 2.0, 8.8 Hz, ArH), 8.06–8.08 (m, 1H, ArH); 13C NMR (CDCl₃+[D₆] DMSO, 75 MHz): δ 39.2, 56.1, 58.6, 60.3, 67.7, 80.1, 104.9, 117.1, 118.5, 120.1, 122.3, 124.6, 125.6, 131.2, 133.2, 137.9, 138.7, 139.3, 139.6, 151.1, 153.4, 153.8, 154.2, 158.1 ppm; IR (KBr) (vmax/cm⁻¹): 1127, 1232, 1285, 1353, 1419, 1461, 1507, 1534, 1617, 3377, 3468; MS (ESI): m/z (578) [M+1]^+; HRMS (ESI m/z) for C₂₇H₂₈N₇O₈ calcd 578.1999, found 578.1996 [M+1]^+. 