Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Electronic supplementary Information

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

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Contents

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 (δ) 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 (ν 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 ^{o}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 ^{o}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 formazon 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 X 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 ^{o}C for 30 min, washed with PBS and incubated with 1mg/ml RNase A solution (Sigma) at 37 ^{o}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 ^{o}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 polyvinylidine 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 ^{o}C overnight (O/N). Active caspase-3 and β-actin was purchased from

Imgenex 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.[57-58] 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.[59] 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.

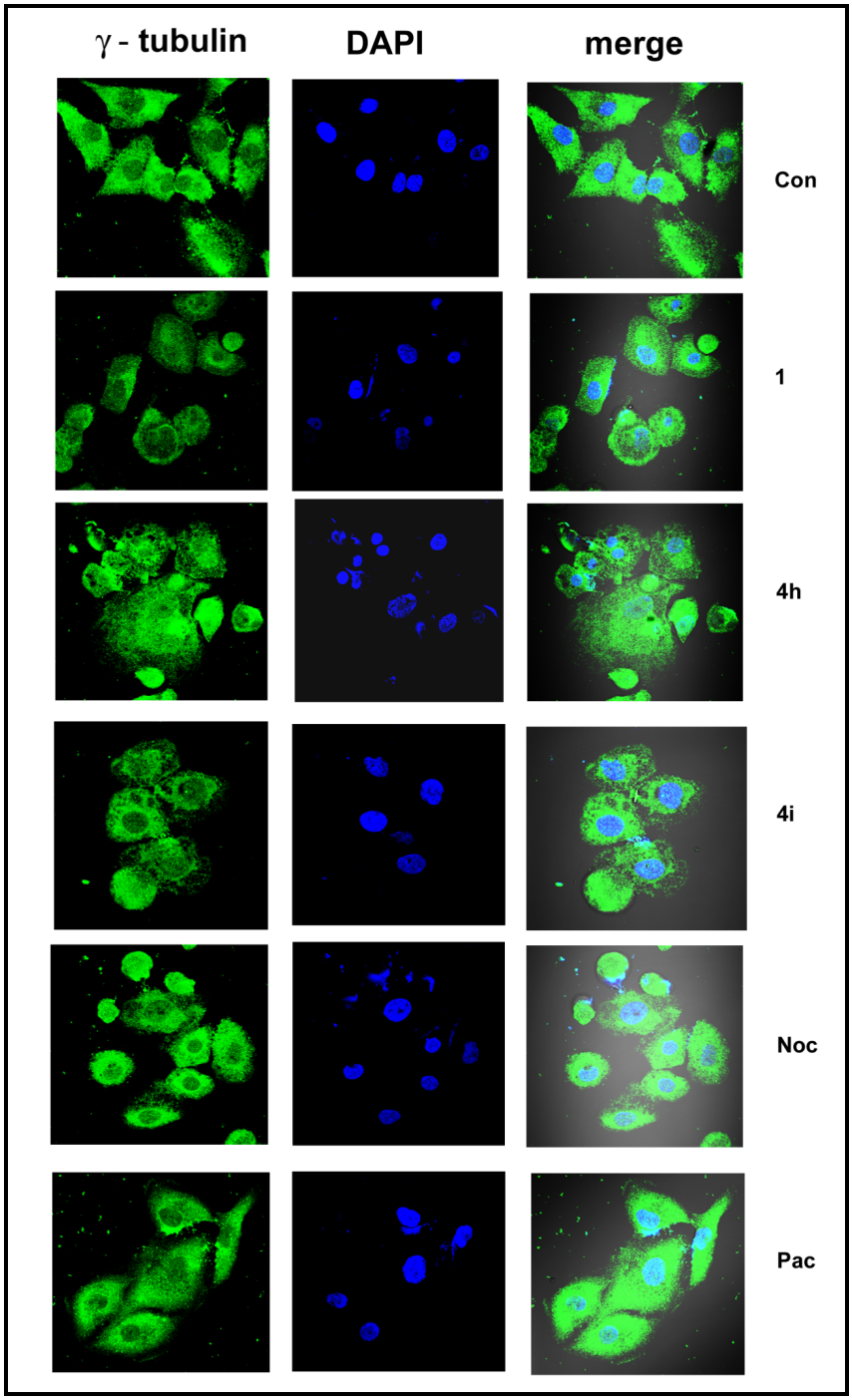

Figure 6: Effect of compound **4h** and **4i** on γ-tubulin. A549 cells were treated with compounds CA-4 (**1**), nocodazole (Noc), paclitaxel (Pac), **4h** and **4i** at 2 μM concentrations for 24 h. γ-Tubulin staining was clearly observed by green colour in control cells and was found to be disrupted 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_2CO_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_2SO_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 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): 5.30 (s, 2H, –C**H**$_2$–Ph), 7.15 (d, 1H, J = 9.0 Hz, Ar**H**), 7.30–7.39 (m, 5H, Ar**H**), 7.82 (dd, 1H, J = 2.8, 9.0 Hz, Ar**H**), 8.21 (d, 1H, J = 2.8 Hz, Ar**H**), 10.76 (s, 1H, –C**H**O); 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_2SO_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 $[M+1]^+$.

*N*1-(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. Triethlamine (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 ^{o}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 $NaHCO_3$ (1 x 20 mL), water (1 x 20 mL) as well as brine (1 x 20 mL) and was dried over anhydrous Na_2SO_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 (5:5) as eluent to afford compound **10** as a pale yellow solid (3.72 g, 85%); mp 195–197 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.83 (s, 6H, 2×–OC**H**$_3$), 3.87 (s, 3H, –OC**H**$_3$), 5.32 (s, 2H, –C**H**$_2$–Ph), 6.94 (s, 2H, Ar**H**), 7.23 (d, 1H, *J* = 8.9 Hz, Ar**H**), 7.34–7.37 (m, 1H, Ar**H**), 7.41 (d, 2H, *J* = 7.6 Hz, Ar**H**), 7.45 (d, 2H, *J* = 6.3 Hz, Ar**H**), 7.78 (s, 1H, –CON**H**), 8.08 (dd, 1H, *J* = 2.5, 8.9 Hz, Ar**H**), 8.35 (d, 1H, *J* = 2.5 Hz, Ar**H**); MS (ESI): *m/z* 439 $[M+1]^+$.

***N*1-(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 Na_2SO_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 (5:5) as eluent to afford compound **11** as a yellow solid (3.45 g, 76%); mp 147–149 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 6H, 2×–OC**H**$_3$), 3.86 (s, 3H, –OC**H**$_3$), 5.30 (s, 2H, –C**H**$_2$–Ph), 6.92 (s, 2H, Ar**H**), 7.22 (d, 1H, *J* = 8.4 Hz, Ar**H**), 7.34-7.36 (m, 1H, Ar**H**), 7.39 (d, 2H, *J* = 6.8

Hz, Ar**H**), 7.42 (d, 2H, *J* = 6.1 Hz, Ar**H**), 7.81 (s, 1H, –CSN**H**), 8.07 (dd, 1H, *J* = 2.6, 8.4 Hz, Ar**H**), 8.34 (d, 1H, *J* = 2.6 Hz, Ar**H**); 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), $NH_2NH_2 \cdot H_2O$ (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 Na_2SO_4. 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)-1*H*-1,2,3,4-tetraazole (13)

To a stirred solution of **12** (2.72 g, 6 mmol) in 5 ml of AcOH, $NaNO_2$ (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 $NaHCO_3$ 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 Na_2SO_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 (4:6) as eluent to afford compound **13** as a pale yellow solid (1.83 g, 74%); mp 158–167 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.81 (s, 6H, 2×–OC**H**$_3$), 3.90 (s, 3H, –OC**H**$_3$), 5.28 (s, 2H, –C**H**$_2$–Ph), 6.61 (s, 2H, Ar**H**), 7.16 (d, 1H, *J* = 8.8 Hz, Ar**H**), 7.31-7.39 (m, 5H, Ar**H**), 7.80 (dd, 1H, *J* = 2.2, 8.8 Hz, Ar**H**), 8.19 (d, 1H, *J* = 2.2 Hz, Ar**H**); MS (ESI): *m/z* 464 $[M+1]^+$.

2-Nitro-4-[1-(3,4,5-trimethoxyphenyl)-1*H*-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), $TiCl_4$ (1.32 g, 7 mmol) was added at 0 ^oC 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 $NaHCO_3$ was added to quench the excess $TiCl_4$ 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 Na_2SO_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 (5:5) as eluent to afford compound **14** as a pale yellow solid (1.67 g, 90%); mp 145–147 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.83 (s, 6H, 2×–OC**H**$_3$), 3.92 (s, 3H, –OC**H**$_3$), 6.60 (s, 2H, Ar**H**), 7.23 (d, 1H, *J* = 9.0 Hz, Ar**H**), 7.89 (dd, 1H, *J* = 2.2, 8.3 Hz, Ar**H**), 8.42 (d, 1H, *J* = 2.2 Hz, Ar**H**); MS (ESI): *m/z* 374 $[M+1]^+$.

5-[4-(Allyloxy)-3-nitrophenyl]-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3,4-tetraazole (15)

To a stirred solution of compound **14** (1.49 g, 4 mmol) and anhydrous K_2CO_3 (1.39 g, 10 mmol) in dry *N,N*-dimethylformamide (5 mL), allyl bromide (0.65 g, 5 mmol) was added at 0 ^oC 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 Na_2SO_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 (4:6) as eluent to afford compound **15** as a pale yellow solid (1.45 g, 88%); mp 115–117 ^oC; ^1H NMR ($CDCl_3$, 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)-1*H*-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 ^{o}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_2SO_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 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.24 (s, 6H, –S(C**H**$_3$)$_2$), 0.95 (s, 9H, –C(C**H**$_3$)$_3$), 3.41–3.54 (m, 2H, isoxazoline–C**H**$_2$), 3.82 (s, 6H, 2×–OC**H**$_3$), 3.83 (s, 3H, –OC**H**$_3$), 3.92 (s, 3H, –OC**H**$_3$), 4.23–4.26 (m, 1H, –OC**H**$_2$), 4.33–4.36 (m, 1H, –OC**H**$_2$), 5.05–5.12 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 6.82 (d, 1H, *J* = 7.8 Hz, Ar**H**), 6.97 (dd, 1H, *J* = 1.9, 7.8 Hz, Ar**H**), 7.19 (d, 1H, *J* = 8.7 Hz, Ar**H**), 7.31 (d, 1H, *J* = 1.9 Hz, Ar**H**), 7.83 (d, 1H, *J* = 8.7 Hz, Ar**H**); MS (ESI): *m/z* 693 $[M+1]^{+}$.

3-(3-(*tert*-Butyldimethylsilyloxy)-4-methoxyphenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxyphenyl)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.24 (s, 6H, –S(C**H**$_3$)$_2$), 0.95 (s, 9H, –C(C**H**$_3$)$_3$), 3.44 (d, 1H, *J* = 5.28 Hz, isoxazoline–C**H**$_2$), 3.47 (d, 1H, *J* = 7.5 Hz, isoxazoline–C**H**$_2$), 3.82 (s, 6H, 2×–OC**H**$_3$), 3.83 (s, 3H, –OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.22–4.28 (m, 1H, –OC**H**$_2$–), 4.31–4.36 (m, 1H, –OC**H**$_2$–), 5.03–5.11 (m, 1H, isoxazoline–C**H**), 6.60 (s, 2H, Ar**H**), 6.82 (d, 1H, *J* = 8.3 Hz, Ar**H**), 7.12 (dd, 1H, *J* = 2.2, 8.3 Hz, Ar**H**), 7.21 (d, 2H, *J* = 2.2 Hz, Ar**H**), 7.83

(dd, 1H, *J* = 2.2, 8.3 Hz, Ar**H**), 8.11 ppm (d, 1H, *J* = 2.2 Hz, Ar**H**); MS (ESI): *m/z* 693 $[M+1]^+$.

3-(3,4-Dimethoxyphenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxy-phenyl)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-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 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.48 (d, 1H, *J* = 1.5 Hz, isoxazoline–C**H**$_2$), 3.50 (d, 1H, *J* = 3.7 Hz, isoxazoline–C**H**$_2$), 3.82 (s, 6H, 2×–OC**H**$_3$), 3.90 (s, 6H, 2×–OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.24–4.29 (m, 1H, –OC**H**$_2$–), 4.32–4.37 (m, 1H, –OC**H**$_2$–), 5.04–5.13 (m, 1H, isoxazoline–C**H**), 6.60 (s, 2H, Ar**H**), 6.83 (d, 1H, *J* = 8.3 Hz, Ar**H**), 7.03 (dd, 1H, *J* = 2.2, 8.3 Hz, Ar**H**), 7.19 (d, 1H, *J* = 9.0 Hz, Ar**H**), 7.33 (d, 1H, *J* = 2.2 Hz, Ar**H**), 7.83 (dd, 1H, *J* = 2.2, 9.0 Hz, Ar**H**), 8.10 (d, 1H, *J* = 2.2 Hz, Ar**H**); MS (ESI): *m/z* 593 $[M+1]^+$.

3-(2,5-Dimethoxyphenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxy-phenyl)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-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 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.60 (d, 2H, *J* = 7.3 Hz, isoxazoline–C**H**$_2$), 3.77 (s, 3H, –OC**H**$_3$), 3.82 (s, 6H, 2×–OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.24–4.34 (m, 2H, –OC**H**$_2$–), 5.00–5.09 (m, 1H, isoxazoline–C**H**), 6.60 (s, 2H, Ar**H**), 6.85 (d, 1H, *J* = 9.0 Hz, Ar**H**), 6.89 (dd, 1H, *J* = 2.8, 9.0 Hz, Ar**H**), 7.23 (d, 2H, *J* = 9.4 Hz, Ar**H**), 7.82 (dd, 1H, *J* = 1.1, 8.8 Hz, Ar**H**), 8.10 (d, 1H, *J* = 2.0 Hz, Ar**H**); MS (ESI): *m/z* 593 $[M+1]^+$.

5-(2-Nitro-4-[1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.52–3.54 (m, 2H, isoxazoline–C**H**$_2$), 3.82 (s, 6H), 3.88 (s, 3H), 3.89 (s, 6H, 2×–OC**H**$_3$), 3.93 (s, 3H, –OC**H**$_3$), 4.30–4.36 (m, 2H, –OC**H**$_2$–), 5.16–5.19 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 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)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-3-(2,3,4-trimethoxyphenyl)-4,5-dihydroisoxa-zole (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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.52–3.54 (m, 2H, isoxazoline–C**H**$_2$), 3.81 (s, 6H, 2×–OC**H**$_3$), 3.88 (s, 3H, –OC**H**$_3$), 3.91 (s, 6H, 2×–OC**H**$_3$), 3.92 (s, 3H, –OC**H**$_3$), 4.30–4.35 (m, 2H, –OC**H**$_2$–), 5.14–5.19 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 6.70 (d, 1H, *J* = 8.9 Hz, Ar**H**), 7.18 (d, 1H, *J* = 8.9 Hz, Ar**H**), 7.46 (d, 1H, *J* = 8.9 Hz, Ar**H**), 7.83 (d, 1H, *J* = 8.9 Hz, Ar**H**), 8.08 (d, 1H, *J* = 1.8 Hz, Ar**H**); MS (ESI): *m/z* 623 $[M+1]^+$.

3-(4-Methoxy-3-nitrophenyl)-5-(2-nitro-4-[1-(3,4,5-trimeth-oxy-phenyl)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-4,5-dihydro-isoxazole (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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.54 (d, 2H, *J* = 8.6 Hz, isoxazoline–C**H**$_2$), 3.82 (s, 6H, 2×–OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.01 (s, 3H, –OC**H**$_3$), 4.28–4.40 (m, 2H, –OC**H**$_2$–), 5.10–5.19 (m, 1H,

isoxazoline–C**H**), 6.60 (s, 2H, Ar**H**), 7.12 (d, 1H, *J* = 8.8 Hz, Ar**H**), 7.17 (d, 1H, *J* = 8.8 Hz, Ar**H**), 7.84 (dd, 1H, *J* = 2.2, 8.8 Hz, Ar**H**), 7.90 (dd, 1H, *J* = 2.2, 8.8 Hz, Ar**H**), 8.02 (d, 1H, *J* = 2.0 Hz, Ar**H**), 8.10 (d, 1H, *J* = 2.0 Hz, Ar**H**); MS (ESI): *m/z* 608 $[M+1]^+$.

3-(2-Fluoro-5-methoxyphenyl)-5-(2-nitro-4-[1-(3,4,5-tri-methoxy-phenyl)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-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 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.55–3.61 (m, 2H, isoxazoline–C$\mathbf{H}_2$), 3.82 (s, 6H, 2×–OC$\mathbf{H}_3$), 3.89 (s, 3H, –OC$\mathbf{H}_3$), 3.92 (s, 3H, –OC$\mathbf{H}_3$), 4.29 (d, 2H, *J* = 4.8 Hz, –OC$\mathbf{H}_3$), 5.05–5.12 (m, 1H, isoxazoline–C**H**), 6.62 (s, 2H, Ar**H**), 7.06–7.14 (m, 2H, Ar**H**), 7.18 (d, 1H, *J* = 9.0 Hz, Ar**H**), 7.35–7.39 (m, 1H, Ar**H**), 7.82 (d, 1H, *J* = 8.6 Hz, Ar**H**), 8.08 (d, 1H, *J* = 2.1 Hz, Ar**H**); MS (ESI): *m/z* 581 $[M+1]^+$.

3-(2-Fluorophenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-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 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.56–3.63 (m, 2H, isoxazoline–C$\mathbf{H}_2$), 3.82 (s, 6H, 2×–OC$\mathbf{H}_3$), 3.90 (s, 3H, –OC$\mathbf{H}_3$), 4.30 (d, 2H, *J* = 4.5 Hz, –OC$\mathbf{H}_2$–), 5.05–5.14 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 7.06-7.14 (m, 1H, Ar**H**), 7.17 (d, 2H, *J* = 9.0 Hz, Ar**H**), 7.35-7.42 (m, 1H, Ar**H**), 7.80 (t, 2H, *J* = 7.5 Hz, Ar**H**), 8.06 (d, 1H, *J* = 2.2 Hz, Ar**H**); MS (ESI): *m/z* 551 $[M+1]^+$.

3-(3-Chlorophenyl)-5-(2-nitro-4-[1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-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** (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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.48 (t, 2H, *J* = 4.53 Hz, isoxazoline–C**H**$_2$), 3.82 (s, 6H, 2×–OC**H**$_3$), 3.89 (s, 3H, –OC**H**$_3$), 4.30 (t, 2H, *J* = 4.5 Hz, –OC**H**$_2$–), 5.06–5.15 (m, 1H, isoxazoline–C**H**), 6.60 (s, 2H, Ar**H**), 7.18 (d, 1H, *J* = 9.0 Hz, Ar**H**), 7.35 (d, 2H, *J* = 6.8 Hz, Ar**H**), 7.52 (d, 1H, *J* = 9.0 Hz, Ar**H**), 7.62 (s, 1H, Ar**H**), 7.81 (dd, 1H, *J* = 2.2, 9.0 Hz, Ar**H**), 8.04 (d, 1H, *J* = 2.2 Hz, Ar**H**); MS (ESI): *m/z* 567 $[M+1]^+$.

5-(2-Nitro-4-[1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3,4-tetraazol-5-yl]phenoxymethyl)-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.40–3.49 (m, 1H, isoxazoline–C**H**$_2$), 3.60–3.70 (m, 1H, isoxazoline–C**H**$_2$), 3.79 (s, 6H, 2×–OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.16 (d, 2H, *J* = 4.5 Hz, –OC**H**$_2$–), 5.09–5.18 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 6.74 (d, 1H, *J* = 8.3 Hz, Ar**H**), 6.81 (dd, 1H, *J* = 1.5, 8.3 Hz, Ar**H**), 7.08 (d, 1H, *J* = 2.2 Hz, Ar**H**), 7.09–7.17 (m, 1H, Ar**H**), 7.19 (d, 1H, *J* = 6.8 Hz, Ar**H**), 7.37–7.45 (m, 1H, Ar**H**), 7.84–7.89 (m, 1H, Ar**H**); MS (ESI): *m/z* 601 $[M+1]^+$.

2-Methoxy-4-(5-((2-nitro-4-(1-(3,4,5-trimethoxyphenyl)-1*H*-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 Na_2SO_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 **17a** as a white solid (580 mg, 91%); mp 189–191 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.43–3.54 (m, 2H, isoxazoline–C**H**$_2$), 3.82 (s, 6H, 2×–OC**H**$_3$), 3.84 (s, 3H, –OC**H**$_3$), 3.92 (s, 3H, –OC**H**$_3$), 4.23–4.25 (m, 1H, –OC**H**$_2$–), 4.33–4.37 (m, 1H, –OC**H**$_2$–), 5.04–5.10 (m, 1H, isoxazoline–C**H**), 6.62 (s, 2H, Ar**H**), 6.82 (d, 1H, *J* = 7.8 Hz, Ar**H**), 6.98 (dd, 1H, *J* = 2.1, 7.9 Hz, Ar**H**), 7.20 (d, 1H, *J* = 8.8 Hz, Ar**H**), 7.31 (d, 1H, *J* = 2.1 Hz, Ar**H**), 7.84 (d, 1H, *J* = 8.9 Hz, Ar**H**); MS (ESI): *m/z* 579 $[M+1]^+$.

2-Methoxy-5-(5-((2-nitro-4-(1-(3,4,5-trimethoxyphenyl)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.45 (d, 1H, *J* = 5.3 Hz, isoxazoline–C**H**$_2$), 3.47 (d, 1H, *J* = 7.5 Hz, isoxazoline–C**H**$_2$), 3.82 (s, 6H, 2×–OC**H**$_3$), 3.84 (s, 3H, –OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.23–4.27 (m, 1H, –OC**H**$_2$–), 4.30–4.36 (m, 1H, –OC**H**$_2$–), 5.03–5.09 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 6.82 (d, 1H, *J* = 8.3 Hz, Ar**H**), 7.13 (dd, 1H, *J* = 2.3, 8.5 Hz, Ar**H**), 7.21 (d, 2H, *J* = 2.3 Hz, Ar**H**), 7.82 (dd, 1H, *J* = 2.3, 8.5 Hz, Ar**H**), 8.10 (d, 1H, *J* = 2.3 Hz, Ar**H**); MS (ESI): *m/z* 579 $[M+1]^+$.

2-(Allyloxy)-5-[1-(3,4,5-trimethoxyphenyl)-1*H*-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 $NaHCO_3$ 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 Na_2SO_4. 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 $[M+1]^+$.

4-[5-(2-Amino-4-[1-(3,4,5-trimethoxyphenyl)-1*H*-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 $NaHCO_3$ 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 Na_2SO_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 **4a** as a white solid (450 mg, 82%); mp 175–178 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.32–3.43 (m, 1H, isoxazoline–C**H**$_2$), 3.55–3.63 (m, 1H, isoxazoline–C**H**$_2$–), 3.81 (s, 6H, 2×–OC**H**$_3$), 3.89 (s, 3H, –OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.21 (d, 2H, *J* = 3.2 Hz, –OC**H**$_2$–), 5.05–5.13 (m, 1H, isoxazoline–C**H**), 6.88 (t, 2H, *J* = 8.1 Hz, Ar**H**), 7.01 (s, 2H, Ar**H**), 7.04 (d, 1H, *J* = 8.5 Hz, Ar**H**), 7.14 (d, 1H, *J* = 8.3 Hz, Ar**H**), 7.24 (s, 1H, Ar**H**), 7.30 (s, 1H, Ar**H**); ^{13}C NMR ($CDCl_3$+[D_6] 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) (ν_{max}/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 $C_{27}H_{28}N_6O_7$ calcd 549.2248, found 549.2253 $[M+1]^+$.

5-[5-(2-Amino-4-[1-(3,4,5-trimethoxyphenyl)-1*H*-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 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.26-3.34 (m, 1H, isoxazoline–C**H**$_2$), 3.46–3.55 (m, 1H, isoxazoline–C**H**$_2$), 3.79 (s, 6H, 2×–OC**H**$_3$), 3.84 (s, 3H, –OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.15 (d, 2H, *J* = 4.5 Hz, –OC**H**$_2$–), 5.05–5.13 (m, 1H, isoxazoline–C**H**), 6.60 (s,

2H, Ar**H**), 6.74 (d, 1H, *J* = 8.5 Hz, Ar**H**), 6.80 (dd, 1H, *J* = 1.9, 8.5 Hz, Ar**H**), 6.84 (d, 1H, *J* = 8.3 Hz, Ar**H**), 7.07 (d, 1H, *J* = 1.9 Hz, Ar**H**), 7.15 (dd, 1H, *J* = 1.9, 8.3 Hz, Ar**H**), 7.27 (s, 1H, Ar**H**); ^{13}C NMR ($CDCl_3$+[D_6] 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 $C_{27}H_{28}N_6O_7$ 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)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.36 (m, 1H, isoxazoline–C**H**$_2$), 3.56 (m, 1H, isoxazoline–C**H**$_2$), 3.77 (s, 6H, 2×–OC**H**$_3$), 3.78 (s, 3H, –OC**H**$_3$), 3.83 (s, 3H, –OC**H**$_3$), 3.84 (s, 3H, –OC**H**$_3$), 4.16 (d, 2H, *J* = 4.3 Hz, –OC**H**$_2$–), 5.03–5.14 (m, 1H, isoxazoline–C**H**), 6.65 (s, 2H, Ar**H**), 7.00 (d, 1H, *J* = 7.5 Hz, Ar**H**), 7.05 (d, 1H, *J* = 8.6 Hz, Ar**H**), 7.11 (dd, 1H, *J* = 1.5, 9.0 Hz, Ar**H**), 7.21 (d, 1H, *J* = 8.5 Hz, Ar**H**),7.32 (d, 1H, *J* = 8.5 Hz, Ar**H**), 7.44 (d, 1H, *J* = 1.5 Hz, Ar**H**); ^{13}C NMR ($CDCl_3$, 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 $C_{28}H_{31}N_6O_7$ 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)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.43–3.49 (m,1H, isoxazoline–C**H**$_2$), 3.60–3.66

(m, 1H, isoxazoline–C**H**$_2$), 3.78 (s, 3H, –OC**H**$_3$), 3.79 (s, 6H, 2×–OC**H**$_3$), 3.81 (s, 3H, –OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.13 (d, 2H, *J* = 4.5 Hz, –OC**H**$_2$–), 5.04–5.12 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 6.72 (d, 1H, *J* = 8.3 Hz, Ar**H**), 6.80 (dd, 1H, *J* = 1.5, 8.3 Hz, Ar**H**), 6.88 (d, 1H, *J* = 9.0 Hz, Ar**H**), 6.93 (dd, 1H, *J* = 3.0, 9.0 Hz, Ar**H**), 7.06 (d, 1H, *J* = 2.2 Hz, Ar**H**), 7.30 (d, 1H, *J* = 3.0 Hz, Ar**H**); ^{13}C NMR ($CDCl_3$, 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) (ν_{max}/cm^{-1}): 1127, 1230, 1294, 1340, 1421, 1461, 1498, 1602, 3365, 3461; MS (ESI): *m/z* 563 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{28}H_{31}N_6O_7$ 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)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.33–3.40 (m, 1H, isoxazoline–C**H**$_2$), 3.50–3.59 (m, 1H, isoxazoline–C**H**$_2$), 3.79 (s, 6H, 2×–OC**H**$_3$), 3.88 (s, 9H, 3×–OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.16 (d, 2H, *J* = 4.5 Hz, –OC**H**$_2$–), 5.10–5.19 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 6.76 (d, 1H, *J* = 8.3 Hz, Ar**H**), 6.81 (dd, 1H, *J* = 1.5, 8.3 Hz, Ar**H**), 6.91 (s, 2H, Ar**H**), 7.08 (d, 1H, *J* = 2.2 Hz, Ar**H**); ^{13}C NMR ($CDCl_3$, 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 $C_{29}H_{33}N_6O_8$ 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)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.37–3.45 (m, 1H, isoxazoline–C**H**$_2$), 3.57–3.66 (m,

1H, isoxazoline–C**H**$_2$), 3.79 (s, 6H, 2×–OC**H**$_3$), 3.86 (s, 3H, –OC**H**$_3$), 3.90 (s, 6H, 2×–OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.15 (d, 1H, *J* = 4.3 Hz, –OC**H**$_2$–), 5.05–5.09 (m, 1H, isoxazoline–C**H**), 6.60 (s, 2H, Ar**H**), 6.68 (d, 1H, *J* = 8.8 Hz, Ar**H**), 6.72 (d, 1H, *J* = 8.5 Hz, Ar**H**), 6.80 (dd, 1H, *J* = 2.0, 8.5 Hz, Ar**H**), 7.06 (d, 1H, *J* = 1.8 Hz, Ar**H**), 7.44 (d, 1H, *J* = 8.6 Hz, Ar**H**); ^{13}C NMR ($CDCl_3$, 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) (ν_{max}/cm^{-1}): 1084, 1127, 1232, 1292, 1357, 1415, 1461, 1500, 1599, 3372, 3457; MS (ESI): *m/z* 593 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{29}H_{33}N_6O_8$ calcd 593.2359, found 593.2375 $[M+1]^+$.

2-[3-(3-Amino-4-methoxyphenyl)-4,5-dihydro-5-isoxazol-yl]meth-oxy-5-[1-(3,4,5-trimethoxyphenyl)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.30–3.39 (m, 1H, isoxazoline–C**H**$_2$), 3.46–3.56 (m, 1H, isoxazoline–C**H**$_2$), 3.79 (s, 6H, 2×–OC**H**$_3$), 3.88 (s, 3H, –OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.14 (d, 2H, *J* = 4.5 Hz, –OC**H**$_2$–), 5.02–5.13 (m, 1H, isoxazoline–C**H**), 6.60 (s, 2H, Ar**H**), 6.75 (d, 1H, *J* = 2.2 Hz, Ar**H**), 6.68–6.80 (m, 2H, Ar**H**), 6.93–6.97 (m, 1H, Ar**H**), 7.06 (d, 1H, *J* = 2.2 Hz, Ar**H**), 7.14 (dd, 1H, *J* = 1.5, 6.0 Hz, Ar**H**); ^{13}C NMR ($CDCl_3$, 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) (ν_{max}/cm^{-1}): 1127, 1235, 1294, 1384, 1420, 1457, 1507, 1601, 3368, 3446; MS (ESI): *m/z* 548 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{27}H_{30}N_7O_6$ calcd 548.2257, found 548.2267 $[M+1]^+$.

2-[3-(2-Fluoro-5-methoxyphenyl)-4,5-dihydro-5-isoxazol-yl]meth-oxy-5-[1-(3,4,5-trimethoxyphenyl)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.41–3.49 (m, 1H, isoxazoline–C**H**$_2$), 3.60–3.67 (m, 1H, isoxazoline–C**H**$_2$), 3.81 (m, 6H, 2×–OC**H**$_3$), 3.90 (s, 3H, –OC**H**$_3$), 3.92 (s, 3H, –OC**H**$_3$), 4.15 (d, 2H, *J* = 4.1 Hz, –OC**H**$_2$–), 5.09–5.18 (m, 1H, isoxazoline–C**H**), 6.61 (s, 2H, Ar**H**), 6.75 (d, 1H, *J* = 8.0 Hz, Ar**H**), 6.80 (dd, 1H, *J* = 1.8, 8.0 Hz, Ar**H**), 7.08-7.17 (m, 3H, Ar**H**), 7.19 (d, 1H, *J* = 6.8 Hz, Ar**H**); ^{13}C NMR ($CDCl_3$+[D_6] DMSO, 75 MHz): δ 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, 138.1, 149.2, 153.1, 153.5, 157.7, 158.3 ppm; IR (KBr) (ν_{max}/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 $C_{27}H_{28}N_6O_6F$ calcd 551.2053, found 521.2038 $[M+1]^+$.

2-[3-(2-Fluorophenyl)-4,5-dihydro-5-isoxazolyl]methoxy-5-[1-(3,4,5-trimethoxy phenyl)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.40–3.49 (m, 1H, isoxazoline–C**H**$_2$), 3.59–3.66 (m, 1H, isoxazoline–C**H**$_2$), 3.79 (m, 6H, 2×–OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.16 (d, 2H, *J* = 4.5 Hz, –OC**H**$_2$–), 5.09–5.18 (m, 1H, isoxazoline–C**H**), 6.60 (s, 2H, Ar**H**), 6.74 (d, 1H, *J* = 8.3 Hz, Ar**H**), 6.80 (dd, 1H, *J* = 1.5, 8.3 Hz, Ar**H**), 7.07 (d, 1H, *J* = 2.2 Hz, Ar**H**), 7.12-7.17 (m, 1H, Ar**H**), 7.19 (d, 1H, *J* = 6.8 Hz, Ar**H**), 7.37–7.45 (m, 1H, Ar**H**), 7.83–7.89 (m, 1H, Ar**H**); ^{13}C NMR ($CDCl_3$+[D_6] DMSO, 75 MHz): δ 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) (ν_{max}/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 $C_{26}H_{26}N_6O_5F$ calcd 521.1948, found 521.1926 $[M+1]^+$.

2-[3-(3-Chlorophenyl)-4,5-dihydro-5-isoxazolyl]methoxy-5-[1-(3,4,5-trimethoxy phenyl)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.32–3.37 (m, 1H, isoxazoline–C**H**$_2$), 3.49–3.55 (m, 1H, isoxazoline–C**H**$_2$), 3.78 (s, 6H, 2×–OC**H**$_3$), 3.90 (s, 3H, –OC**H**$_3$), 4.16 (d, 2H, *J* = 4.2 Hz, –OC**H**$_2$–), 5.13–5.18 (m, 1H, , isoxazoline–C**H**$_2$), 6.60 (s, 2H, Ar**H**), 6.74 (d, 1H, *J* = 8.3 Hz, Ar**H**), 6.80 (d, 1H, *J* = 8.3 Hz, Ar**H**), 7.07 (s, 1H, Ar**H**), 7.35 (t, 1H, *J* = 8.3 Hz, Ar**H**), 7.39 (d, 1H, *J* = 7.5 Hz, Ar**H**), 7.56 (d, 1H, *J* = 7.5 Hz, Ar**H**), 7.66 (s, 1H, Ar**H**); ^{13}C NMR ($CDCl_3$+[D_6] 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 $C_{26}H_{26}N_6O_5Cl$ 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)-1*H*-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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.41–3.46 (m, 1H, isoxazoline–C**H**$_2$), 3.59–3.64 (m, 1H, isoxazoline–C**H**$_2$), 3.78 (s, 6H, 2×–OC**H**$_3$), 3.85 (s, 3H, –OC**H**$_3$), 4.21 (d, 2H, *J* = 3.9 Hz, –OC**H**$_2$–), 5.15–5.24 (m, 1H, isoxazoline–C**H**), 6.69 (s, 2H, Ar**H**), 6.72 (d, 1H, *J* = 8.0 Hz, Ar**H**), 6.81 (d, 1H, *J* = 8.3 Hz, Ar**H**), 7.09 (s, 1H, Ar**H**), 7.71 (d, 2H, *J* = 7.5 Hz, Ar**H**), 7.85 (d, 2H, *J* = 6.9 Hz, Ar**H**); ^{13}C NMR ($CDCl_3$+[D_6] 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 $C_{27}H_{26}N_6O_5F_3$ calcd 571.1916, found 571.1921 $[M+1]^+$.

2-[3-(4-Methoxy-3-nitrophenyl)-4,5-dihydro-5-isoxazolyl]-methoxy-5-[1-(3,4,5-trimethoxyphenyl)-1*H*-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 Et_3N (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 ^{o}C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.31–3.41 (m, 1H, isoxazoline–C**H**$_2$), 3.49–3.62 (m, 1H, isoxazoline–C**H**$_2$), 3.71 (s, 3H, –OC**H**$_3$), 3.79 (s, 3H, –OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 4.02 (s, 3H, –OC**H**$_3$), 4.19 (d, 1H, *J* = 4.3 Hz, –OC**H**$_2$–), 4.24 (d, 1H, *J* = 4.3 Hz, –OC**H**$_2$–), 5.15–5.24 (m, 1H, isoxazoline–C**H**), 6.57 (s, 1H, Ar**H**), 6.61 (s, 1H, Ar**H**), 6.82 (s, 1H, Ar**H**), 7.17 (dd, 2H, *J* = 2.4, 8.8 Hz, Ar**H**), 7.34 (d, 1H, *J* = 9.0 Hz, Ar**H**), 7.98 (dd, 1H, *J* = 2.0, 8.8 Hz, Ar**H**), 8.06–8.08 (m, 1H, Ar**H**); ^{13}C NMR ($CDCl_3$+[D_6] 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) (ν_{max}/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 $C_{27}H_{28}N_7O_8$ calcd 578.1999, found 578.1996 $[M+1]^+$.