Electronic Supplementary Information

Discovery of a 3,4,5-Trisubstituted-1,2,4-Triazole Agonist with High Affinity and Selectivity at the Somatostatin Subtype-4 (sst_4) Receptor

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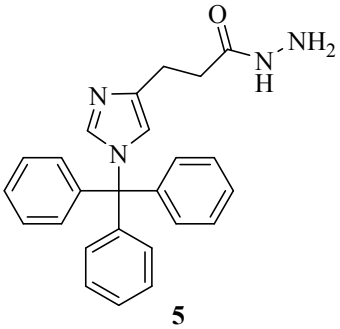
2. Experimental

2.1. General procedures

Flash chromatography was performed either on a Biotage SPI apparatus using Si columns, size 25-S, 25-M, 40-S, 40-M (Biotage, Inc.) or with Ace Glass columns using Sorbent Technology silica gel (230-400 mesh). Melting points were recorded on either an Opti-Melt (Stanford Research System) or Mel-Temp-3.0 electrothermal apparatus (Barnstead International, Dubuque, IA). IR spectra were obtained on a Varian 800 FT-IR instrument. Reaction mixtures and purities of final products were analyzed with a LC/MS-2010A (Shimadzu). NMR spectra were recorded on a JEOL ECS-400, 400 MHz spectrometer. Chemical shifts are reported in parts per million (δ) relative to the internal standard tetramethylsilane. NMR data are reported using the following abbreviations: broad (br), singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), and multiplet (m). Elemental analyses were obtained from Atlantic Microlab, Inc., Norcross, GA. Analyses indicated by the symbols of the elements were within ± 0.4 % of the theoretical values. High resolution mass spectra (HRMS) were obtained from the Notre Dame Science Mass Spectroscopy and Proteomics Facility, Norte Dame, IN.

1.2. Synthesis

1.2.1. 3-(1-Trityl-1*H*-imidazol-4-yl)propanehydrazide **(5)**.

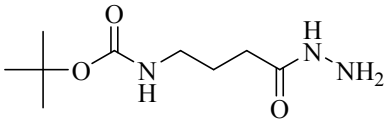


5

Methyl 3-(1-trityl-1*H*-imidazol-4-yl)propanoate[1] (3.0 g, 7.6 mmol) and 51% hydrazine hydrate (3 g, 8.2 mL, 7.6 mmol) in MeOH (45 mL) were heated to reflux overnight under nitrogen. Evaporation of the solvent under reduced pressure gave a yellow, sticky semisolid.

Absolute EtOH (2 x 25 mL) was added and then evaporated to yield a white paste. The solid was washed with H_2O (50 mL) and Et_2O (50 mL) and placed in a vacuum oven to dry. Recrystallization from EtOAc gave 3.86 (95%) of **5**: mp 177-179 ^oC; IR 3235, 1674, 1628 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.05 (br s, 1H, N*H*), 2.55 (t, *J* = 7 Hz, 2 H, CH_2CH_2CONH), 2.83 (t, *J* = 7 Hz, 2H), 3.85 (br s, 2H, NH_2), 6.57 (s, 1H), 7.10 (m, 5H), 7.32 (m, 10H), 8.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.00 (CH_2CH_2CONH), 34.70 (CH_2CH_2CONH), 75.39 (Ph_3C), 118.57, 128.17, 129.81, 138.41, 139.67, 142.34, 173.73 ($CONHNH_2$). Anal. Calcd for $C_{25}H_{24}N_4O$ (C, H, N).

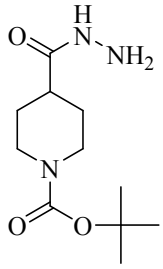
1.2.2. *tert*-Butyl 4-hydrazinyl-4-oxobutylcarbamate **(6)**.



6

Following the general procedure described for **5**, methyl 4-(*tert*-butoxycarbonylamino)butanoate[2] (20.13 g, 92.7 mmol) and 80% hydrazine hydrate (61 mL) in EtOH (250 mL) gave, after trituration with hexanes, 17.31 g (86%) of a white solid: mp 114-116 ^oC (lit[2], no reported mp); ^1H NMR ($CDCl_3$) δ 1.44 (s,9H), 1.80 (p, 2H), 2.22 (t, *J* = 7 Hz, 2H), 3.17 (m, 2H), 3.68 (br, 2H), 4.76 (br s, 1H), 7.71 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.61, 28.46, 31.39, 39.58, 79.95, 156.73, 173.54.

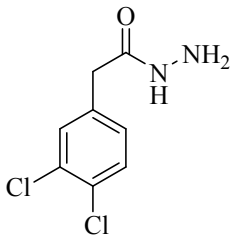
1.2.3. *tert*-Butyl 4-(hydrazinecarbonyl)piperidine-1-carboxylate **(7)**.



7

In a similar manner as described for **5**, ethyl 1-(*tert*-butoxycarbonyl)-4-piperidine[3] and 80% hydrazine hydrate (20 mL) in EtOH (80 mL) gave, after trituration with hexanes, 6.66 g (100%) of a white solid: mp 106-108 ^{o}C (lit[4], mp 105 ^{o}C); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.64 (ddd, J = 16, 12, 4 Hz, 4H), 1.77 (d, J = 12 Hz, 2H), 2.21 (m, 1H), 2.72 (br s, 2H), 4.13 (b, 2H), 6.87 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.44, 28.50, 41.65, 43.00, 79.50, 154.71, 175.30.

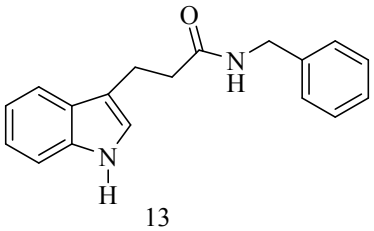
1.2.4. 2-(3,4-Dichlorophenyl)acetohydrazide **(8)**.



8

Using the general method, methyl 2-(3,4-dichlorophenyl)acetate (3.86 g, 17.6 mmol) and 80 % hydrazine hydrate (11 mL) in MeOH (50 mL) yielded 3.53 g (91%) of a white solid: mp 134-135 ^{o}C (lit[5], no reported mp); ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 2H, CH_2CO), 3.83 (br s, 2H, NHNH_2, 6.74 (br s, 1H) 7.10 (dd, J = 8 Hz, 1H), 7.36 (d, J = 2 Hz, 1H) 7.40 (d, J = 8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 40.72, 128.76, 130.91, 131.30, 131.86, 133.02, 134.12, 170.55.

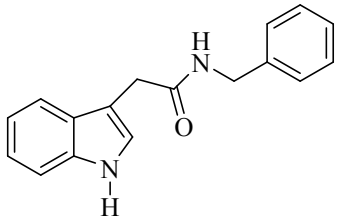
1.2.5. *N*-Benzyl-3-(1*H*-indol-3-yl)propanamide **(13)**.



13

3-(1*H*-Indol-3-yl)propanoic acid **(10**, 5.0 g, 26.4 mmol**)** was dissolved in dry THF (200 mL) and added to a 500 mL, 3-necked flask cooled to 0-5 ^{o}C under a nitrogen atmosphere. The cooled solution was treated with *N*-methylmorpholine (NMM, 2.64 g, 2.87 mL, 26.4 mmol) followed by the addition of isobutyl chloroformate (IBCF, 3.56 g, 3.38 mL, 26.4 mmol) over 3 min. During the addition of IBCF, a white precipitate formed. Benzylamine (2.79 g, 2.84 mL, 26.4 mmol) in dry THF (50 mL) was added dropwise over 5 min, and the reaction mixture was allowed to warm to room temperature and stir for an additional 5-10 min. The precipitate was filtered, and the filtrate was evaporated under reduced pressure to yield a gummy yellow solid. The solid was dissolved in CH_2Cl_2 (70 mL) and was washed successively with 5% NaOH (3 x 60 mL), water (50 mL), 5% HCl (3 x 50 mL), and water (50 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated under reduced pressure to afford a solid. Recrystallization from CH_2Cl_2/hexanes gave 6.20 g (84%) of white crystals: mp 123-124 ^{o}C (lit[6], mp 125-126.5 ^{o}C). ^{1}H NMR ($CDCl_3$) δ 2.59 (t, *J* = 7.6 Hz, 2H), 3.13 (t, *J* = 7.6 Hz 2H), 4.33 (d, *J* = 5.6 Hz, 2H), 5.67 (s, 1H), 6.92 (s, 1H), 7.05 (d, *J* = 8 Hz, 2H), 7.10 (t, *J* = 8 Hz, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 7.25 (m, 3H), 7.35 (d, *J* = 7.6, Hz, 1H), 7.58 (d, *J* = 7.6 Hz, 1H), 8.20 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.35, 37.37, 43.46, 111.36, 114.81, 118.78, 119.43, 122.02, 122.12, 127.15, 127.47, 127.76, 128.69, 136.42, 138.26, 172.71.

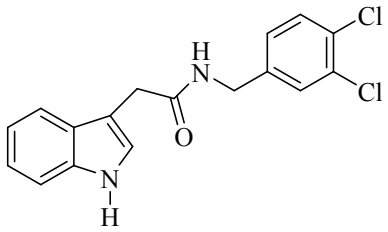
1.2.6. *N*-Benzyl-2-(1*H*-indol-3-yl)acetamide **(11)**.



11

Using the method described for the synthesis of **13**, 2-(1*H*-indol-3-yl)acetic acid (**9**, 3.00 g, 17.1 mmol), benzylamine (1.83 g, 1.87 mL, 17.1 mmol), NMM (1.73 g, 1.88 mL, 17.1 mmol), and IBCF (2.34 g, 2.22 mL, 17.1 mmol) in THF (100 mL) gave 3.42 g (76%) of **11** as white crystals after recrystallization from EtOH/Et_2O: mp 154-155 ^oC (lit[7], 156-158 ^oC); ^1H NMR ($CDCl_3$) δ 3.81 (s, 2H), 4.40 (d, J = 6 Hz, 2H), 6.02 (t, 1H), 7.13 (m, 4H), 7.25 (m, 4H), 7.38 (d, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 33.52, 43.44, 108.93, 111.53, 118.87, 120.21, 122.79, 123.89, 127.05, 127.36, 127.48, 128.63, 136.46, 138.28, 171.60.

1.2.7. *N*-(3,4-Dichlorobenzyl)-2-(1*H*-indol-3-yl)acetamide **(12)**.

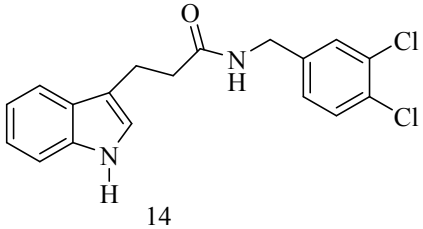


12

Following the method described for the synthesis of **13**, 2-(1*H*-indol-3-yl)acetic acid (**9**, 3.00 g, 17.1 mmol), 3,4-dichlorobenzylamine (3.01 g, 2.28 mL, 17.1 mmol), NMM (1.73 g, 1.88 mL, 17.1 mmol), and IBCF (2.34 g, 2.22 mL, 17.1 mmol) in THF (100 mL) gave 3.42 g (71%) of **12** as white crystals after recrystallization from EtOH/H_2O: mp 165-167 ^oC (lit[8], mp 158-162 ^oC); ^1H NMR ($CDCl_3$) δ 3.82 (s, 2H), 4.31 (d, J = 6 Hz, 2H), 6.05 (t, 1H), 6.95 (dd, J = 3, 1 Hz, 1H), 7.18 (m, 3H), 7.28 (m, 2H), 7.42 (d, J = 8 Hz, 1H), 7.52 (d, J = 8 Hz, 1H), 8.27 (s, 1H); ^{13}C

NMR ($CDCl_3$) δ 33.46, 42.24, 108.81, 111.59, 118.77, 120.45, 122.98, 123.91, 126.79, 127.10, 129.28, 130.51, 131.32, 132.82, 136.46, 138.73, 171.90.

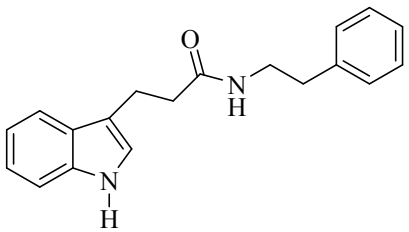
1.2.8. *N*-(3,4-Dichlorobenzyl)-3-(1*H*-indol-3-yl)propanamide **(14)**.



14

In a similar manner as described for the synthesis of **13**, the acid **10** (3.00 g, 15.9 mmol), 3,4-dichlorobenzylamine (2.80 g, 2.11 mL, 15.9 mmol), NNM (1.61 g, 1.75 mL, 15.9 mmol), and IBCF (2.17 g, 2.05 mL, 15.9 mmol) in THF (100 mL) gave 4.07 g (74%) of white crystals after recrystallization from EtOH/H_2O: mp 98-100 ^oC; NMR ($CDCl_3$) δ 2.61 (t, J = 7 Hz, 2H), 3.13 (t, J = 7 Hz, 2H), 4.24 (d, J = 6 Hz, 2H), 5.72 (t, 1H), 6.81 (d, J = 2 Hz, 1H), 6.93 (m, 1H), 7.11 (m, 2H), 7.19 (m, 1H), 7.25 (d, J = 8 Hz, 1H), 7.35 (d, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 8.08 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.33, 37.29, 42.23, 111.32, 114.47, 118.56, 119.37, 121.91, 122.09, 126.82, 126.90, 129.26, 130.37, 131.16, 132.37, 136.27, 138.56, 172.73. HRMS (TOF MS ES^+) calcd for $C_{18}H_{17}Cl_2N_2O$ $(M+H)^+$ 347.0718; Found 347.0712. Anal. Calcd for $C_{18}H_{16}Cl_2N_2O$ (C,H,N).

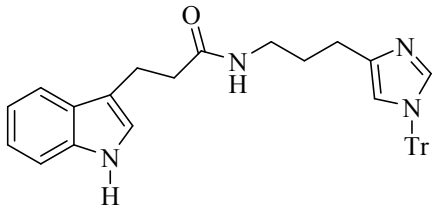
1.2.9. 3-(1*H*-Indol-3-yl)-*N*-phenethylpropanamide **(15)**.



15

Following the method described for the synthesis of **13**, acid **10** (3.00 g, 15.9 mmol), 2-phenethylamine (1.93 g, 1.99 mL, 15.9 mmol), NMM (1.61 g, 1.75 mL, 15.9 mmol), and IBCF (2.17 g, 2.05 mL, 15.9 mmol) in THF (100 mL) gave a white solid after trituration with Et_2O. Recrystallization from EtOH/H_2O afforded 3.51 g (76%) of white crystals: mp 87-88 °C (lit[6], 88-89 °C); ^{1}H NMR ($CDCl_3$) δ 2.52 (t, *J* = 8 Hz, 2H), 2.65 (t, *J* = 8 Hz, 2H), 3.09 (t, *J* = 8 Hz, 2H), 3.44 (q, *J* = 6 Hz, 2H), 5.36 (br s, 1H), 6.94 (d, *J* = 8 Hz, 1H), 6.99 (s, 2H), 7.12 (t, *J* = 8 Hz, 1H), 7.23 (m, 4H), 7.37 (d, *J* = 8 Hz, 1H), 7.59 (d, *J* = 8 Hz, 1H), 8.09 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.30, 35.54, 37.34, 40.43, 111.37, 114.92, 118.80, 119.43, 121.91, 122.16, 126.52, 127.15, 128.69, 128.82, 136.40, 138.97, 172.75.

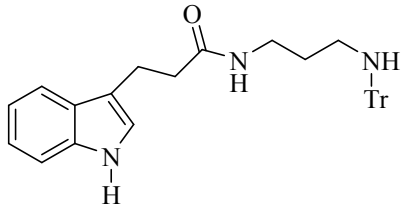
1.2.10. 3-(1*H*-Indol-3-yl)-*N*-(3-(1-trityl-1*H*-imidazol-4-yl)propyl)propanamide **(16)**.



16

In a similar manner as described for the synthesis of **13**, acid **10** (1.42 g, 7.5 mmol), 3-(1-trityl-1*H*-imidazol-4-yl)propane-1-amine[9] (2.77 g, 7.5 mmol), NMM (0.76 g, 0.82 mL), and IBCF (1.02 g, 0.98 mL, 7.5 mmol) in THF (170 mL) gave 2.40 g (59%) of **16** after recrystallization from CH_2Cl_2/hexanes: mp 130-148 °C; ^{1}H NMR ($CDCl_3$) δ 1.66 (m, 2H), 2.36 (t, *J* = 7 Hz, 2H), 3.08 (t, *J* = 7 Hz, 2H), 3.19 (t, *J* = 7 Hz, 2H), 6.04 (br s, 1H), 6.45 (s, 1H), 6.96 (s, 1H), 7.11 (m, 10H), 7.55 (d, *J* = 8 Hz, 1H), 8.38 (br s, 1H) ; ^{13}C NMR ($CDCl_3$) δ 21.67, 25.53, 28.93, 37.66, 39.06, 75.24, 111.30, 114.93, 118.18, 118.73, 119.26, 121.94, 122.21, 127.15, 128.14, 129.83, 136.46, 138.27, 140.93, 149.50, 172.95. ESI-HRMS (TOF, MS, ES$^+$) calcd for $C_{36}H_{35}N_4O$ $(M + H)^+$ 539.2811; Found: 539.2805.

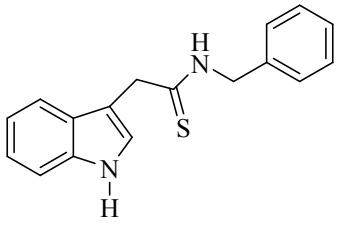
1.2.11. 3-(1*H*-Indol-3-yl)-*N*-(3-(tritylamino)propyl)propanamide **(17)**.



17

Using the general method described for **13**, acid **10** (2.42 g, 12.8 mmol), *N*-tritylpropane-1,3-diamine[10] (4.05 g, 12.8 mmol), NMM (1.29 g, 1.40 mL, 12.8 mmol), and IBCF (1.75 g, 1.66 mL, 12.8 mmol) in THF (120 mL) gave 3.9 (63%) of a white solid: mp 183-185 ^{o}C; ^{1}H NMR (DMSO-d_6) δ 1.84 (m, 2H), 2.28 (t, J = 8 Hz, 2H), 2.74 (m, 4H), 3.00 (q, J = 6 Hz, 2H), 6.92 (t, J = 7 Hz, 1H), 7.01 (t, J = 7 Hz, 2H), 7.29 (d, J = 7 Hz, 1H), 7.42 (m, 15H), 8.04 (t, J = 6 Hz, 1H), 10.38 (br s, 2H), 10.76 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.70, 26.98, 36.14, 36.77, 43.68, 75.48, 111.89, 114.17, 118.65, 118.78, 121.43, 122.57, 127.44, 129.10, 129.43, 136.75, 139.71, 173.08. ESI-HRMS (TOF, MS, ES$^+$) calcd for $C_{33}H_{34}N_3O$ $(M + H)^+$ 488.2702; Found 488.2696.

1.2.12. *N*-Benzyl-2-(1*H*-indol-3-yl)ethanethioamide **(18)**.

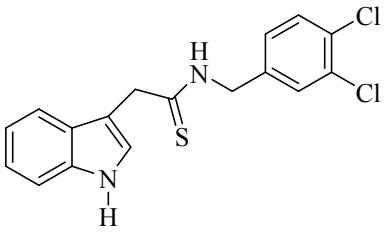


18

Using the general method described for **20**, amide **11** (5.5 g, 20.8 mmol) and Lawesson's reagent (4.61 g, 11.4 mmol) in THF (150 mL) afforded a crude product which was purified on silica gel flash chromatography using CH_2Cl_2-hexanes (50:50) followed by CH_2Cl_2 as the eluents. Recrystallization from CH_2Cl_2/hexanes gave 5.27 g (90%) of pink crystals: mp 102-104 ^{o}C; IR 1605 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.37 (s, 2H), 4.80 (d, J = 6 Hz, 2H), 7.05 (m, 2H),

7.11 (s, 1H), 7.15 (m, 1H), 7.21 (m, 3H), 7.24 (m, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.54 (d, *J* = 8 Hz, 1H), 7.62 (br s, 1H), 8.29 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 43.19, 49.84, 108.57, 111.64, 118.96, 120.50, 123.08, 124.46, 126.87, 127.69, 127.85, 128.79, 136.08, 136.50, 202.48. Anal. Calcd for $C_{17}H_{16}N_2S$ (C,H,N).

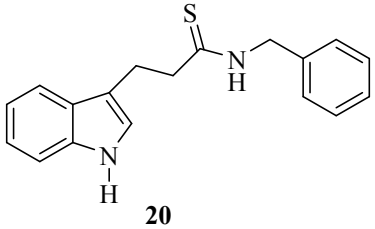
1.1.13. *N*-(3,4-Dichlorobenzyl)-2-(1*H*-indol-3-yl)ethanethioamide **(19)**.



19

In a similar manner as described for **20**, amide **12** (5.5 g, 16.5 mmol) and Lawesson's reagent (3.73 g, 9.22 mmol) in THF (200 mL) gave, after silica gel flash chromatography using CH_2Cl_2-hexanes (50:50) followed by CH_2Cl_2 as the eluents, a solid product. Recrystallization from CH_2Cl_2-hexanes yielded 4.87 g (85%) of pink crystals: mp 138-140.5 ^{o}C; IR 1520 (C=S) cm^{-1}; ^{1}H NMR δ 4.38 (s, 2H), 4.77 (d, *J* = 5 Hz, 2H), 6.91 (dd, *J* = 3, 1 Hz, 1H), 7.15 (m, 3H), 7.25 (m, 2H), 7.40 (d, *J* = 8 Hz, 1H), 7.53 (d, *J* = 8 Hz, 1H), 7.59 (s, 1H), 8.24 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 43.19, 48.20, 108.54, 111.68, 118.89, 120.76, 123.30, 124.45, 126.77, 127.02, 129.49, 130.63, 131.82, 132.79, 136.42, 136.51, 203.18. Anal. Calcd for $C_{17}H_{14}Cl_2N_2S$ (C,H,N).

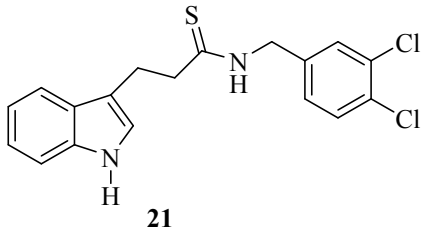
1.2.14. *N*-Benzyl-3-(1*H*-indol-3-yl)propanethioamide **(20)**.



20

A mixture of the amide **13** (3.5 g, 12.6 mmol) and Lawesson's reagent (2.80 g, 6.92 mmol) in THF (200 mL) was refluxed for 2h. The solvent was removed under reduced pressure, and the crude product was purified by silica gel flash chromatography using CH_2Cl_2/hexanes (50:50) followed by CH_2Cl_2 as the eluents. Recrystallization from CH_2Cl_2 gave 3.67 g (81%) of white crystals: mp 99-100 ^oC; IR 1536 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.04 (t, *J* = 8 Hz, 2H), 3.27 (t, *J* = 8 Hz, 2H), 4.61 (d, *J* = 5 Hz, 2H), 6.91 (m, 2H), 6.99 (m, 2H), 7.11 (t, 8 Hz, 1H), 7.23 (m, 4H), 7.26 (d, *J* = 8 Hz, 1H), 7.60 (d, *J* = 8 Hz, 1H), 7.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.21, 47.97, 50.42, 111.37, 114.23, 118.81, 119.70, 122.32, 127.03, 128.02, 128.21, 128.85, 136.00, 136.39, 204.67. Anal. Calcd for $C_{18}H_{18}N_2S$ (C,H,N).

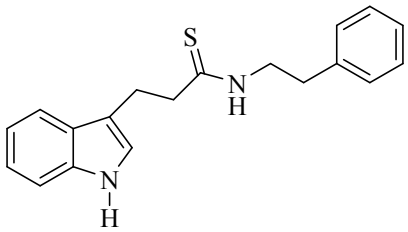
1.2.15. *N*-(3,4-Dichlorobenzyl)-3-(1*H*-indol-3-yl)propanethioamide **(21)**.



21

Using the general method described for **20**, amide **14** (3.79 g, 10.9 mmol) and Lawesson's reagent (2.65 g, 6.55 mmol) in THF (100 mL) gave a crude product that was purified by silica gel flash chromatography using CH_2Cl_2/hexanes (50:50) followed by CH_2Cl_2 as the eluents. Recrystallization from CH_2Cl_2/hexanes afforded 3.24 g (82%) of white crystals: mp 100-103 ^oC; IR 1520 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.05 (t, *J* = 7 Hz, 2H), 3.26 (t, *J* = 7 Hz, 2H), 4.54 (d, *J* = 5 Hz, 2H), 6.69 (dd, *J* = 3,1 Hz, 1H), 6.92 (br 1H), 6.96 (m, 2H), 7.10 (t, *J* = 8 Hz, 1H), 7.18 (t, *J* = 8 Hz, 1H), 7.23 (m, 1H), 7.36 (d, *J* = 8 Hz,1H), 7.59 (d, *J* = 8 Hz, 1H), 7.97 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.22, 47.93 (CH_2CH_2CSNH), 48.77 111.58, 114.00, 118.69, 119.77, 122.40, 122.42, 126.90, 127.47, 129.83, 130.63, 131.96, 132.65, 136.36, 205.46 (*C*SNH). Anal. Calcd for $C_{18}H_{16}Cl_2N_2S$ (C,H,N).

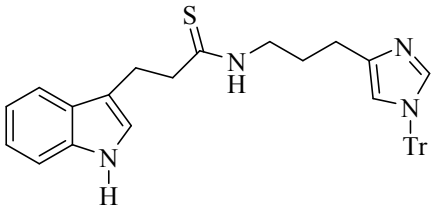
1.2.16. 3-(1*H*-Indol-3-yl)-*N*-phenethylpropanethioamide **(22)**.



22

In a similar manner as described for **20**, amide **15** (1.3 g, 4.45 mmol) and Lawesson's reagent (1.08 g, 2.67 mmol) in THF (80 mL) gave a solid product after flash chromatography on silica gel using CH_2Cl_2/hexanes (50:50) as the eluent. Recrystallization from CH_2Cl_2/hexanes yielded 1.17 g (85%) of yellow crystals: mp 111-112 ^oC; IR (C=S) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.65 (t, *J* = 7 Hz, 2H), 2.96 (t, *J* = 8 Hz, 2H), 3.22 (t, *J* = 8 Hz, 2H), 3.75 (q, *J* = 8 Hz, 2H), 6.84 (br s, 1H), 6.90 (m, 3H), 7.21 (m, 5H), 7.37 (d, *J* = 8 Hz, 1H), 7.58 (d, *J* = 8 Hz, 1H), 7.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.02, 33.69, 46.77, 47.90, 111.41, 118.79, 119.66, 122.20, 122.35, 126.75, 127.03, 128.64, 128.81, 136.37, 138.17, 204.79. Anal. Calcd for $C_{19}H_{20}N_2S$ (C,H,N).

1.2.17. 3-(1*H*-Indol-3-yl)-*N*-(3-(1-trityl-1*H*-imidazol-4-yl)propyl)propanethioamide **(23)**.

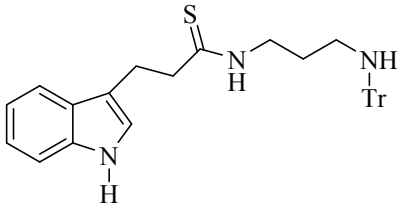


23

Using the general method described for **20**, amide **16** (2.20 g, 4.08 mmol) and Lawesson's reagent (1.65 g, 4.06 mmol) in THF (200 mL) afforded a crude reaction product. Silica gel flash chromatography using CH_2Cl_2/MeOH/NH_4OH (97:2:1) as the eluent gave 1.4 g (50%) of a white foam: IR 1529 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.78 (m, 2H), 2.40 (t, *J* = 6 Hz, 2H), 2.99 (t, *J* = 7 Hz, 2H), 3.23 (t, *J* = 7 Hz, 2H), 3.57 (q, *J* = 5 Hz, 2H), 6.47 (s, 1H), 6.92 (m, 2H), 7.10 (m,

7H), 7.32 (m, 11H), 7.54 (d, J = 8 Hz, 1H), 8.31 (s, 1H), 9.29 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.41, 25.89, 26.81, 46.58, 47.60, 76.80, 111.32, 114.59, 118.42, 118.75, 119.28, 121.93, 122.20, 127.16, 128.20, 129.82, 136.39, 138.11, 140.54, 142.42, 203.93. HRMS (TOF MS ES$^+$) calcd for $C_{36}H_{35}N_4S$ $(M + H)^+$ 555.2582; Found: 555.2577.

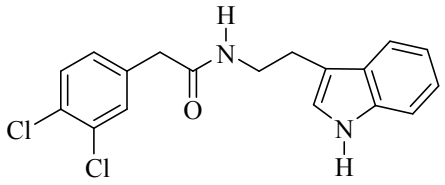
1.2.18. 3-(1*H*-Indol-3-yl)-*N*-(3-(tritylamino)propyl)propanethioamide **(24)**.



24

Using the general procedure described for **20**, amide **17** (2.96 g, 6.07 mmol) and Lawesson's reagent (2.40 g, 5.92 mmol) in THF (630 mL) gave a crude reaction product. Flash chromatography on silica gel using CH_2Cl_2 followed by CH_2Cl_2/MeOH/NH_4OH (97:2:1) as the eluents afforded 1.85 (61%) of a white foam: IR 1528 (C=S) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (m, 2H), 2.08 (t, J = 8 Hz, 2H), 3.00 (t, J = 8 Hz, 2H), 3.24 (t, J = 8 Hz, 2H), 3.63 (q, J = 6 Hz, 2H), 6.89 (d, J = 1.2 Hz, 1H), 7.17 (m, 4H), 7.25 (m, 8H), 7.35 (m, 6H), 7.54 (d, J = 8 Hz, 1H), 7.76 (s, 1H), 7.91 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.20, 28.40, 42.07, 45.38, 48.19, 71.22, 111.22, 114.48, 118.95, 119.57, 122.11, 122.22, 126.58, 127.11, 128.01, 128.64, 136.22, 145.61, 204.16. HRMS (TOF MS ES$^+$) calcd for $C_{33}H_{34}N_3S$ $(M + H)^+$ 504.2473; Found: 504.2468.

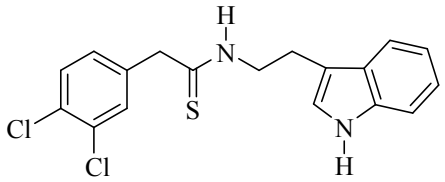
1.2.19. *N*-(2-(1*H*-Indol-3-yl)ethyl)-2-(3,4-dichlorophenyl)acetamide **(25)**.



25

2-(3,4-Dichlorophenyl)acetic acid (5.0 g, 24.4 mmol) was dissolved in CH_2Cl_2 (60 mL) and stirred in an ice bath. Thionyl chloride (8.70 g, 24.4 mmol) in CH_2Cl_2 (25 mL) was added dropwise to the reaction mixture under a nitrogen atmosphere. After the addition of thionyl chloride, the reaction mixture was warmed to room temperature and stirred overnight. The solvent was evaporated under reduced pressure, and additional CH_2Cl_2 (2 x 50 mL) was added and evaporated to remove the excess thionyl chloride. The resulting solid was dissolved in THF (100 mL), Et_3N (2.46 g, 3.42 mL, 24.3 mmol) was added, and the solution was cooled in an ice bath. Tryptamine (3.91 g, 24.4 mmol) in THF (50 mL) was added dropwise, and the reaction mixture was allowed to come to room temperature and stir for 6 h. The solution was filtered to remove the precipitated Et_3N hydrochloride, and the solvent was evaporated under reduced pressure to give a brick-orange solid. The product was dissolved in CH_2Cl_2 and washed successively with 1N HCl (3 x 60 mL), H_2O (60 mL), and 1N NaOH (3 x 60 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated. Recrystallization of the crude product from CH_2Cl_2 gave 0.78 g (10%) of yellow crystals: mp 105-108 ^oC; IR 1629 (C=O); ^1H NMR ($CDCl_3$) δ 2.92 (t, J = 6 Hz, 2H), 3.38 (s, 2H), 3.55 (q, J = 6 Hz, 2H), 5.46 (br s, 1H), 6.85 (d, J = 2 Hz, 1H), 6.93 (dd, J = 8, 2 Hz, 1H), 7.11 (t, J = 7 Hz, 1H), 7.25 (m, 3H), 7.35 (d, J = 8 Hz, 1H), 7.53 (d, J = 8 Hz, 1H), 8.13 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.0, 39.94, 42.78, 111.48, 112.54, 118.65, 119.65, 122.23, 122.39, 127.19, 128.88, 130.77, 131.30, 131.43, 132.72, 135.08, 136.42, 169.81. Anal. Calcd for $C_{18}H_{16}Cl_2N_2O$ (C,H,N).

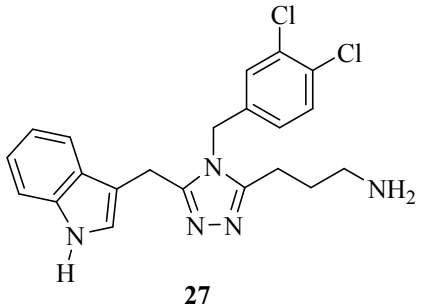
1.2.20. *N*-(2-(1*H*-Indol-3-yl)ethyl)-2-(3,4-dichlorophenyl)ethanethioamide **(26)**.



26

Following the general procedure as described for **20**, amide **25** (2.8 g, 8.06 mmol) and Lawesson's reagent (1.88 g, 4.65 mmol) in THF (80 mL) gave a crude reaction product. Flash chromatography on silica gel using CH_2Cl_2/hexanes (50:50) followed by CH_2Cl_2 as the eluents yielded 2.42 g (80%) of cream-colored crystals after recrystallization from CH_2Cl_2/hexanes: mp 120-122 ^oC; IR 1618 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.04 (t, J = 6 Hz, 2H), 3.90 (s, 2H), 3.95 (q, J = 6 Hz, 2H), 6.77 (s, 1H), 6.83 (d, J = 8 Hz, 1H), 6.88 (s, 1H), 7.10 (m, 1H), 7.21 (d, J = 8 Hz, 2H), 7.38 (d, J = 8 Hz, 1H), 7.51 (d, J = 8 Hz, 1H), 7.97 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.88, 52.00, 111.57, 111.79, 118.59, 119.92, 122.28, 122.69, 126.85, 128.84, 130.90, 131.26, 131.86, 132.92, 135.09, 136.47, 200.16. Anal. Calcd for $C_{18}H_{16}Cl_2N_2S$ (C,H,N).

1.2.21. General Method for the Synthesis of Triazoles: Synthesis of 3-(5-((1*H*-Indol-3-yl)methyl)-4- (3,4-dichlorobenzyl)-4*H*-1,2,4-triazol-3-yl)propan-1-amine **(27)**.

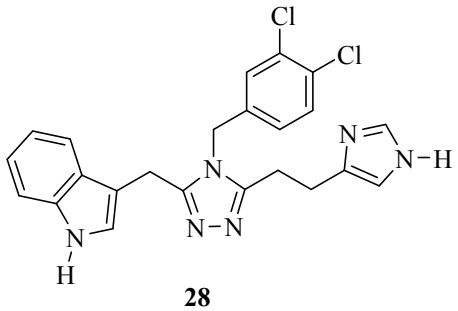


27

N-(3,4-Dichlorobenzyl)-2-(1*H*-indol-3-yl)ethanethioamide (**19**, 1.4 g, 4.01 mmol) and *tert*-butyl 4-hydrazinyl-4-oxobutylcarbamate (**6**, 1.17 g, 5.39 mmol) were dissolved in CH_2Cl_2 (70 mL) and treated with silver benzoate (1.84 g, 8.04 mmol) and glacial HOAc (0.67 mL, 12.0 mmol). Upon the addition of the silver benzoate and HOAc, the solution turned black. The reaction was stirred under nitrogen, and the reaction mixture was analyzed by LC/MS periodically for 24 h. The solvent was evaporated under reduced pressure, and the reaction product was purified by silica gel flash chromatography using first EtOAc followed by elution of the desired compound with EtOAc/MeOH/NH_4OH (90:9:1). The Boc-protected product was

refluxed overnight in a mixture of EtOH (30 mL) and 1N HCl (20 mL). After removal of the solvents under reduced pressure, the resulting residue was treated with water (50 mL) and extracted with CH_2Cl_2 (3 x 60 mL). The aqueous phase was basified with 2 N NaOH and extracted with CH_2Cl_2 (3 x 60 mL). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give a yellow solid. Recrystallization from CH_2Cl_2 gave 0.640 g (39%) of a cream-colored solid: mp 168-169 °C; ^{1}H NMR ($CDCl_3$) δ 1.85 (m, 2H), 2.59 (t, *J* = 7 Hz, 2H), 2.72 (t, *J* = 7 Hz, 2H), 4.20 (s, 3H), 4.83 (s, 2H), 6.40 (dd, *J* = 8, 2 Hz, 1H), 6.68 (d, *J* = 2 Hz, 1H), 6.89 (s, 1H), 7.04 (t, *J* = 8 Hz, 1H), 7.15 (m, 2H), 7.27 (d, *J* = 8 Hz, 1H), 8.43 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.33, 30.41, 41.26, 45.42, 109.45, 111.37, 118.69, 120.05, 122.64, 122.74, 126.73, 127.48, 130.62, 132.08, 133.09, 134.87, 136.26, 153.80, 155.17. HRMS (TOF MS ES$^+$) calcd for $C_{21}H_{22}Cl_2N_5$ $(M + H)^+$ 414.1252; Found 414.1247. Anal. Calcd for $C_{21}H_{21}Cl_2N_5$ (C,H,N).

1.2.22. 3-((5-(2-(1*H*-Imidazol-4-yl)ethyl)-4-(3,4-dichlorobenzyl)-4*H*-1,2,4-triazol-3-yl)methyl-1*H*-indole **(28)**.

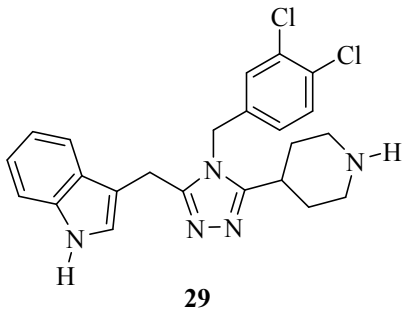


28

Following the method described for **27**, thioamide **19** (1.40 g, 4.01 mmol), hydrazide **5** (1.91 g, 4.82 mmol), silver benzoate (1,84 g, 8.02 mmol), and glacial HOAc (0.70 g, 12.1 mmol) in CH_2Cl_2 (100 mL) was stirred under a nitrogen atmosphere for 7 h. The crude product was purified by silica gel chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1) as the eluents. The purified trityl-protected intermediate was refluxed overnight in a mixture of

absolute EtOH (60 mL) and 1N HCl (60 mL). After acid-base extraction, the product was purified by silica gel flash chromatography using CH_2Cl_2 followed by a gradient solvent system starting with CH_2Cl_2/MeOH/NH_4OH (98:1:1) to CH_2Cl_2/MeOH/NH_4OH (84:15:1) to yield 0.600 g (33%) of a yellow foam: ^{1}H NMR ($CDCl_3$) δ 3.01 (s, 4H), 4.13 (s, 2H), 4.69 (s, 2H), 6.54 (br s, 1H), 6.80 (s, 1H), 7.08 (t, *J* = 8 Hz, 1H), 7.18 (t, *J*= 8 Hz, 1H), 7.24 (br s, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.45 (d, *J* = 8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 22.19, 24.92, 45.47, 109.05, 111.61, 118.37, 119.79, 122.18, 122.56, 125.12, 126.58, 127.76, 130.95, 132.42, 133.38, 134.96, 135.28, 153.85, 155.90. HRMS (TOF MS ES$^+$) calcd for $C_{23}H_{21}Cl_2N_6$ $(M + H)^+$ 451.1205; Found 451.1199.

1.2.23. 3-((4-(3,4-Dichlorobenzyl)-5-(piperidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-1*H*-indole **(29)**.

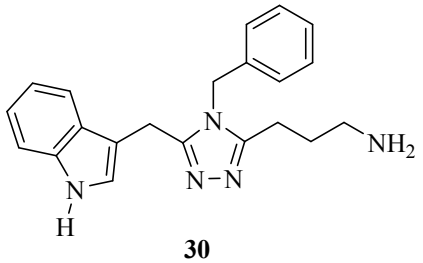


29

Following the method described for **27**, thioamide **19** (1.4 g, 4.01 mmol), hydrazide **7** (1.17 g, 4.81 mmol), silver benzoate (1.84 g, 8.04 mmol), and glacial HOAc (0.67 mL, 12.1 mmol) in CH_2Cl_2 (100 mL) were stirred for 7 h under a nitrogen atmosphere. Flash chromatography on silica gel using EtOAc followed by EtOAc/MeOH/NH_4OH (90:9:1) as the eluents yielded the Boc-protected intermediate. Removal of the Boc-protecting group by refluxing in a mixture of EtOH (40 mL) and 1N HCl (65 mL) afforded a solid. An acid-base work-up followed by trituration of the resulting solid with CH_2Cl_2 gave 0.900 g (51%) of a white solid: mp 196-199 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 1.70 (m, 2H), 1.90 (m, 2H), 2.56 (m, 3H), 3.14 (d, *J* = 3 Hz, 2H), 4.19 (s, 2H), 4.85 (s, 2H), 6.40 (dd, *J* = 8, 2.4 Hz, 1H), 6.69 (d, *J* = 2.4 Hz, 1H), 7.05

(t, J = 8 Hz, 1H), 7.16 (m, 2H), 7.27 (d, J = 8 Hz, 1H), 7.43 (d, J = 8 Hz, 1H), 8.22 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.30, 31.93, 45.26, 46.32, 109.52, 111.31, 118.69, 120.09, 122.65, 122.74, 124.51, 126.73, 127.35, 130.63, 132.12, 133.14, 135.05, 136.18, 153.62, 158.25. HRMS (TOF MS ES$^+$) calcd for $C_{23}H_{24}Cl_2N_5$ (M + H)$^+$ 440.1409; Found: 440.1403.

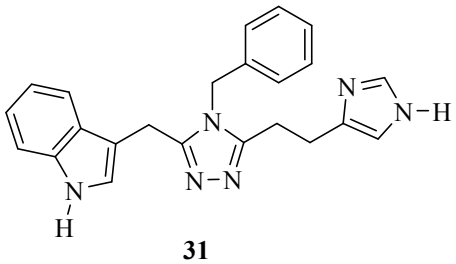
1.2.24. 3-(5-((1*H*-Indol-3-yl)methyl)-4-benzyl-4*H*-1,2,4-triazol-3-yl)propan-1-amine **(30)**.



30

Using the general procedure described for **27**, thioamide **18** (1.5 g, 5.35 mmol), hydrazide **6** (1.39 g, 6.40 mmol), silver benzoate (2.45 g, 10.5 mmol), and glacial HOAc (0.92 mL, 15.7 mmol) in CH_2Cl_2 (40 mL) afforded a crude reaction product. Flash chromatography on silica gel using EtOAc followed by EtOAc/MeOH/NH_4OH (90:9:1) gave the Boc-protected intermediate. Deprotection using a mixture of EtOH (40 mL) and 1N HCl (40 mL) followed by an acid-base work-up gave 0.740 g (40%) of a cream-colored solid after recrystallization from CH_2Cl_2: mp 147-150 ^{o}C; ^{1}H ($CDCl_3$) δ 1.83 (m, 2H), 2.62 (t, J = 8 Hz, 2H), 2.70 (t, J = 8 Hz, 2H), 4.18 (s, 2H), 4.89 (s, 2H), 6.76 (m, 2H), 6.91 (s, 1H), 7.05 (t, J = 8 Hz, 1H), 7.16 (t, J = 8 Hz, 1H), 7.25 (m, 3H), 7.32 (d, J = 8 Hz, 1H), 7.48 (d, J = 8 Hz, 1H), 8.42 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.38, 22.48, 30.53, 41.35, 46.42, 109.78, 111.32, 118.97, 119.85, 122.46, 122.76, 125.74, 126.91, 128.11, 129.10, 134.97, 136.37, 153.84, 155.22. HRMS (TOF MS ES$^+$) calcd for $C_{21}H_{24}N_5$ (M + H)$^+$ 346.2032; Found: 346.2026.

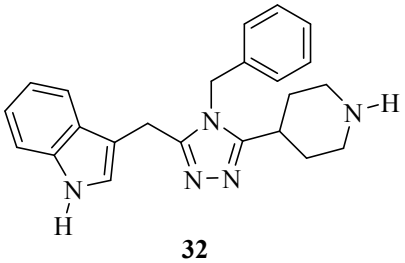
1.2.25. 3-((5-2-(1*H*-Imidazol-4-yl)ethyl-4-benzyl-4*H*-1,2,4-triazol-3-yl)methyl)-1*H*-indole **(31)**.



31

In a manner similar to **27**, thioamide **18** (1.50 g, 5.35 mmol), hydrazide **5** (2.5 g, 6.31 mmol), silver benzoate (2.45 g, 10.7 mmol), and glacial HOAc (0.92 mL, 16.0 mmol) in CH_2Cl_2 (80 mL) were stirred under nitrogen for 27 h. The trityl-protected intermediate was purified by silica gel flash chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1) as the solvents. Removal of the trityl group was accomplished by refluxing overnight in a mixture of absolute EtOH (30 mL) and 1N HCl (50 mL). After acid-base work-up, the residue was triturated with CH_2Cl_2 to yield 0.921 g (50%) of a white solid: ^{1}H NMR (DMSO-d_6) δ 2.71 (s, 4H), 4.10 (s, 2H), 5.07 (s, 2H), 6.78 (br s, 2H), 6.89 (t, J = 7 Hz, 1H), 7.01 (t, J = 7 Hz, 1H), 7.12 (s, 1H), 7.19 (s, 3H), 7.27 (d, J = 8 Hz, 1H), 7.42 (d, J = 2.4 Hz, 2H), 10.85 (1H), 11.71 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.05, 25.32, 26.00, 46.06, 109.08, 111.91, 112.48, 118.97, 119.14, 121.68, 124.15, 125.13, 126.64, 127.33, 128.04, 129.19, 135.10, 136.57, 136.81, 139.88, 153.69, 154.68. HRMS (TOF MS ES$^+$) calcd for $C_{23}H_{23}N_6$ $(M + H)^+$ 383.1984; Found: 383.1979.

1.2.26. 3-((4-Benzyl-5-(piperidin-4-yl)-4*H*-1,2,4-triazol-3-yl)methyl)-1*H*-indole **(32)**.

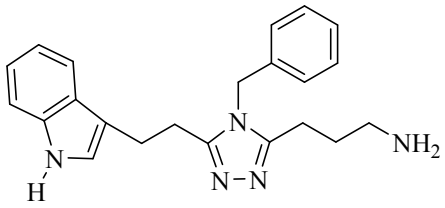


32

Following the general procedure described for **27**, thioamide **18** (1.50 g, 5.35 mmol), hydrazide **7** (1.56 g, 6.41 mmol), silver benzoate (2.45 g, 10.7 mmol), and glacial HOAc (0.92

mL, 16.0 mmol) in CH_2Cl_2 (30 mL) were stirred for 168 h. The crude reaction product was purified by silica gel flash chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (90:9:1). The Boc-protected triazole was refluxed overnight in a mixture of EtOH (50 mL) and 1N HCl (30 mL). The solvent was evaporated under reduced pressure, and the residue was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (3 x 60 mL). The aqueous layer was basified with 2 N NaOH and extracted with CH_2Cl_2 (3 x 60 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and evaporated under reduced pressure to yield 0.77 g (38%) of a white solid: mp 202-204 ^oC; ^1H NMR ($CDCl_3$) δ 1.67 (d, J = 13 Hz, 2H), 1.84 (m, 2H), 2.55 (m, 3H), 3.10 (d, J = 13 Hz, 2H), 4.13 (s, 2H), 4.88 (s, 2H), 6.77 (m, 2H), 6.91 (s, 1H), 7.04 (t, J = 7 Hz, 1H), 7.16 (t, J = 7 Hz, 1H), 7.26 (m, 3H), 7.32 (d, J = 8 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 8.55 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.31, 31.94, 33.59, 46.27, 46.38, 109.67, 111.33, 118.96, 119.80, 122.38, 122.88, 125.69, 126.93, 128.14, 129.13, 135.19, 136.37, 153.69, 158.37. HRMS (TOF MS ES^+) calcd for $C_{23}H_{26}N_5$ $(M + H)^+$ 372.2188; Found 372.2183.

1.2.27. 3-(5-(2-(1*H*-Indol-3-yl)ethyl)-4-benzyl-4*H*-1,2,4-triazol-3-yl)propan-1-amine **(33)**.

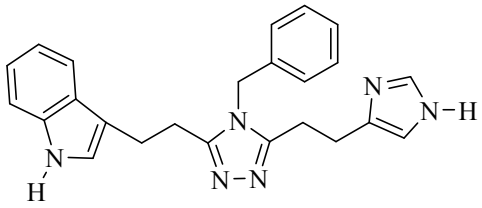


33

Thioamide **20** (1.00 g, 3.40 mmol), hydrazide **6** (1.25 g, 5.75 mmol), and mercuric acetate (1.14 g, 3.58 mmol) were dissolved in THF (150 mL) and stirred under a nitrogen atmosphere for 50 h. The solvent was evaporated to give an oily residue. The residue was dissolved in CH_2Cl_2, and the CH_2Cl_2 was washed with $NaHSO_4$ (3 x 60 mL) and H_2O (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. Flash column chromatography on silica

gel eluting with EtOAc followed with EtOAc/MeOH/NH_4OH (95:4:1) gave the Boc-protected intermediate. The intermediate was dissolved in a mixture of EtOH (30 mL) and 1N HCl (20 mL) and was refluxed overnight, and the EtOH removed under reduced pressure. The resulting liquid was diluted with H_2O (50 mL), and the acidic solution was extracted with CH_2Cl_2 (3 x 60 mL). The CH_2Cl_2 extracts were discarded, and the aqueous phase was basified with 2 N NaOH and extracted with CH_2Cl_2 (3 x 60 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give a solid. Recrystallization from CH_2Cl_2/hexanes gave 0.197 g (16%) of cream-colored crystals: mp 170-173 ^oC; ^1H NMR ($CDCl_3$) δ 1.78 (m, 2H), 2.60 (t, *J* = 7 Hz, 2H), 2.98 (t, *J* = 8 Hz, 2H), 3.22 (t, *J* = 8 Hz, 2H), 4.65 (s, 2H), 6.77 (m, 2H), 6.93 (s, 1H), 7.04 (t, *J* = 8 Hz, 1H), 7.16 (t, *J* = 8 Hz, 1H), 7.25 (m, 3H), 7.34 (d, *J* = 8 Hz, 2H), 8.39 (br s, 1H); ^{13}C NMR δ 22.46, 23.78, 26.35, 30.66, 41.29, 45.97, 111.33, 114.64, 118.56, 119.50, 122.13, 125.73, 127.15, 128.22, 129.19, 135.02, 136.29, 154.26, 155.07. HRMS (TOF MS ES^+) calcd for $C_{22}H_{26}N_5$ $(M + H)^+$ 360.2188; Found 360.2183.

1.2.28. 3-(2-(5-(2-(1*H*-Imidazol-4-yl)ethyl)-4-benzyl-4*H*-1,2,4-triazol-3-yl)ethyl)-1*H*-indole **(34)**.

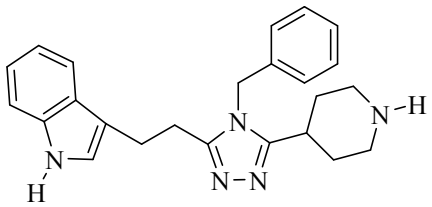


34

N-Benzyl-3-(1*H*-indol-3-yl)propanethioamide (**20**, 1.2 g, 4.07 mmol), hydrazide **5** (3.22 g, 8.12 mmol), and mercuric acetate (1.43 g, 4.49 mmol) were dissolved in THF (170 mL) and stirred under a nitrogen atmosphere for 29 h. During the reaction, the solution turned from yellow to dark brown. The solvent was evaporated, and the crude material was dissolved in

CH_2Cl_2. The CH_2Cl_2 phase was washed with $NaHSO_4$ (3 x 60 mL) and H_2O (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel, eluting first with EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1). The trityl-intermediate was dissolved in a mixture of EtOH (100 mL) and 1 N HCl (24 mL) and refluxed overnight under nitrogen. After removal of the EtOH, H_2O (50 mL) was added. The acidic solution was extracted with CH_2Cl_2 (3 x 60 mL), and the CH_2Cl_2 were discarded. The aqueous acid phase was basified with 2 N NaOH, and extracted with CH_2Cl_2 (3 x 60 mL). After drying over Na_2SO_4, the solvent was evaporated under reduced pressure to yield a yellow foam. Flash chromatography on silica gel using CH_2Cl_2 followed by CH_2Cl_2/MeOH/NH_4OH (80:19:1) as the eluents yielded 0.150 g (11%) of a white foam: ^1H NMR ($CDCl_3$) δ 2.81 (t, *J* = 6 Hz, 2H), 2.98 (t, *J* = 7 Hz, 2H), 3.04 (t, *J*= 6 Hz, 2H), 3.20 (t, *J* = 7 Hz, 2H), 4.53 (s, 2H), 6.65 (s, 1H), 6.70 (m, 2H), 6.82 (d, 1H), 7.01 (t, *J* = 8 Hz, 1H), 7.15 (t, *J* = 8 Hz, 1H), 7.23 (m, 3H), 7.35 (t, *J* = 5 Hz, 2H), 7.48 (s, 1H), 8.77 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.18, 23.69, 25.45, 26.17, 45.97, 111.48, 114.03, 118.41, 119.42, 120.30, 122.12, 122.27, 125.77, 127.08, 128.30, 129.23, 133.40, 134.56, 134.78, 136.36, 154.62, 155.29. HRMS (TOF MS ES$^+$) calcd for $C_{24}H_{25}N_6$ $(M + H)^+$ 397.2141; Found: 397.2135

1.2.29. 3-(2-(4-Benzyl-5-(piperidin-4-yl)-4*H*-1,2,4-triazol-3-yl)ethyl)-1*H*-indole **(35)**.

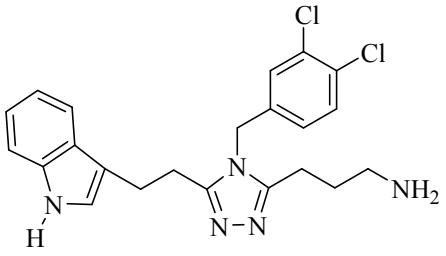


35

Thioamide **20** (0.77 g, 2.61 mmol) and hydrazide **7** (1.27 g, 5.22 mmol) were dissolved in THF (150 mL) followed by the addition of mercuric acetate (0.92 g, 2.89 mmol). The mixture

was stirred under a nitrogen atmosphere for 48 h, and the solvent was evaporated to give the Boc-protected intermediate. The crude product was dissolved in CH_2Cl_2 (70 mL), and the CH_2Cl_2 was washed with $NaHSO_4$ (3 x 60 mL) and H_2O (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. Flash column chromatography on silica gel using EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1) as the solvents afforded the purified intermediate. Removal of the Boc-protecting group was accomplished by refluxing in a mixture of EtOH (10 mL) and 1 N HCl (10 mL). The solvents were evaporated, and H_2O (50 mL) was added. The aqueous solution was basified with 2 N NaOH and extracted with CH_2Cl_2 (3 x 60 mL). After evaporation of the CH_2Cl_2 , the residue was purified by silica gel flash chromatography using CH_2Cl_2 followed by a gradient solvent system of CH_2Cl_2/MeOH/NH_4OH (98:1:1) to CH_2Cl_2/MeOH/NH_4OH (74:25:1) to yield 0.140 g (14%) of a cream-colored foam: ^{1}H NMR ($CDCl_3$) δ 1.70 (d, *J* = 12 Hz, 2H), 1.83 (m, 2H), 2.61 (t, *J* = 12 Hz, 3H), 2.96 (t, *J* = 8 Hz, 2H), 3.17 (m, 4H), 4.68 (s, 2H), 6.76 (m, 2H), 6.97 (s, 1H), 7.01 (t, *J* = 8 Hz, 1H), 7.15 (t, *J* = 8 Hz, 1H), 7.25 (m, 4H), 7.34 (d, *J* = 8 Hz, 1H), 8.44 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.70, 26.29, 31.18, 32.94, 45.80, 45.86, 111.37, 114.51, 118.48, 119.48, 122.06, 125.65, 127.16, 128.30, 129.26, 135.11, 136.26, 154.89, 157.42. HRMS (TOF MS ES$^+$) calcd for $C_{24}H_{28}N_5$ $(M + H)^+$ 386.2345; Found 386.2339.

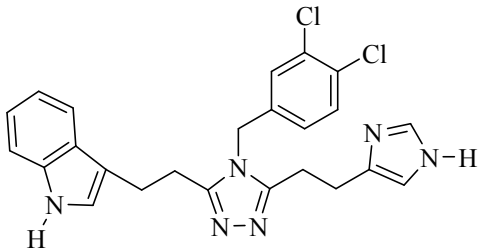
1.2.30. 3-(5-(2-(1*H*-Indol-3-yl)ethyl)-4-(3,4-dichlorobenzyl)-4*H*-1,2,4-triazol-3-yl)propan-1-amine **(36)**.



36

In a similar manner as described for **27**, thioamide **21** (1.50 g, 4.13 mmol), hydrazide **6** (1.13 g, 5.20 mmol), silver benzoate (1.89 g, 8.25 mmol), and glacial HOAc (0.74 mL, 12.4 mmol) in CH_2Cl_2 (80 mL) were stirred for 168 h under a nitrogen atmosphere. The reaction product was purified by flash chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (90:9:1). The Boc-protecting group was removed by refluxing in a mixture of EtOH (100 mL) and 1 N HCl (40 mL). EtOH was removed under reduced pressure, and the resulting aqueous solution was basified with 2 N NaOH and extracted with CH_2Cl_2 (3 x 60 mL). After drying (Na_2SO_4), evaporation of the CH_2Cl_2 under reduced pressure gave 0.740 g (42%) of a white solid: mp 183-186 ^oC; ^1H NMR ($CDCl_3$) δ 1.78 (m, 2H), 2.58 (t, *J* = 7 Hz, 2H), 2.71 (t, *J* = 7 Hz, 2H), 2.95 (t, *J* = 7 Hz, 2H), 3.23 (t, *J* = 7 Hz, 2H), 4.48 (s, 2H), 6.44 (dd, *J* = 8, 2.4 Hz, 1H), 6.80 (s, 1H), 6.99 (s, 1H), 7.05 (t, *J* = 8 Hz, 1H), 7.17 (t, *J* = 8 Hz, 1H), 7.20 (s, 1H), 7.25 (m, 4H) Hz, 4H), 8.42 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.38, 23.94, 26.31, 30.63, 41.21, 44.74, 111.43, 114.31, 118.39, 119.61, 122.25, 124.79, 127.03, 127.62, 131.10, 132.44, 133.47, 135.17, 136.28, 154.29, 154.92. HRMS (TOF MS ES^+) calcd for $C_{22}H_{24}Cl_2N_5$ $(M + H)^+$ 428.1409; Found: 428.1403.

1.2.31. 3-(2-(5-(2-(1*H*-Imidazol-4-yl)ethyl)-4-(3,4-dichlorobenzyl)-4*H*-1,2,4-triazol-3-yl)ethyl)-1*H*-indole **(37)**.

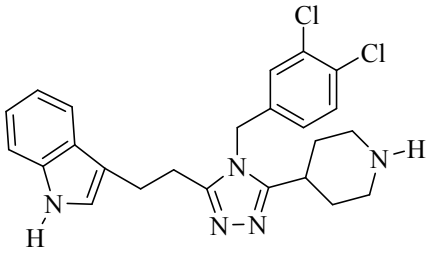


37

A mixture of thioamide **21** (1.49 g, 4.10 mmol), hydrazide **5** (3.22 g, 8.12 mmol), and mercuric acetate (1.43 g, 4.49 mmol) were dissolved in THF (170 mL) and stirred under a

nitrogen atmosphere for 96 h. The solvent was evaporated to give an oil. The residue was dissolved in CH_2Cl_2 (70 mL), and the solution was washed with $NaHSO_4$ (3 x 60 mL) and H_2O (50 mL). The CH_2Cl_2 was dried (Na_2SO_4) and evaporated under reduced pressure. The trityl-protected intermediate was purified by flash chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1) as the eluents. The trityl group was removed by refluxing in a mixture of EtOH (140 mL) and 1 N HCl (35 mL). EtOH was evaporated, and the acidic solution was extracted with CH_2Cl_2 (3 x 60 mL). The CH_2Cl_2 extracts were discarded, and the aqueous phase was basified with 2 N NaOH and extracted with CH_2Cl_2 (3 x 60 mL). After evaporation of the CH_2Cl_2, the resulting residue was purified by flash chromatography using CH_2Cl_2 followed by a gradient solvent system starting from CH_2Cl_2/MeOH/NH_4OH (98:1:1) to CH_2Cl_2/MeOH/NH_4OH (84:15:1) to yield 0.350 (18%) of a white foam: ^{1}H NMR ($CDCl_3$) δ 2.81 (t, *J* = 6 Hz, 2H), 2.95 (t, *J* = 7 Hz, 2H), 3.04 (t, *J* = 6 Hz, 2H), 3.20 (t, *J* = 7 Hz, 2H), 4.32 (s, 2H), 6.35 (dd, *J* = 8, 2.4 Hz, 1H), 6.64 (s, 1H), 6.71 (d, *J* = 8 Hz, 1H), 6.79 (m, 1H), 7.01 (t, *J* = 8 Hz, 1H), 7.16 (t, *J* = 8 Hz, 1H), 7.21 (d, *J* = 8 Hz, 2H), 7.31 (d, *J* = 8 Hz, 1H), 7.35 (d, *J* = 8 Hz, 1H), 7.48 (s, 1H), 8.92 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.93, 25.32, 26.15, 44.73, 111.58, 113.70, 118.27, 119.54, 122.23, 122.39, 124.86, 126.94, 127.67, 131.11, 132.50, 133.43, 134.80, 134.88, 136.37, 154.47, 155.14. HRMS (TOF MS ES$^+$) calcd for $C_{24}H_{23}Cl_2N_6$ $(M + H)^+$ 465.1361; Found: 465.1356.

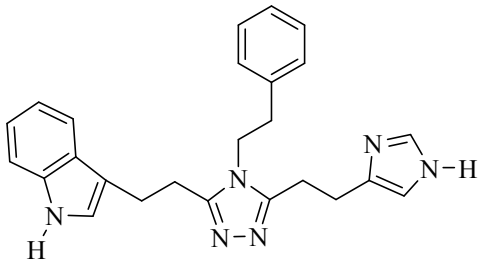
1.2.32. 3-(2-(4-(3,4-Dichlorobenzyl)-5-(piperidin-4-yl)-4*H*-1,2,4-triazol-3-yl)ethyl)-1*H*-indole **(38)**.



38

Following the method described for **27**, thioamide **21** (0.60 g, 1.65 mmol), hydrazide **7** (0.50 g, 2.06 mmol), silver benzoate (0.79 g, 3.45 mmol), and glacial HOAc (0.30 g, 5.18 mmol) in CH_2Cl_2 (40 mL) were stirred under a nitrogen atmosphere for 48 h. The Boc-protected intermediate was purified by silica gel flash chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1) as the eluents. The purified intermediate was refluxed overnight in a mixture of EtOH (40 mL) and 1N HCl (20 mL). After the standard acid-base work-up, trituration with CH_2Cl_2 and acetone yielded 0.252 g (34%) of **38** as a cream-colored solid: mp 242-249 ^oC; ^1H NMR (DMSO-d_6) δ 1.65 (m, 4H), 2.59 (t, 2H), 2.82 (m, 3H), 3.01 (m, 4H), 5.13 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.87 (t, J = 7 Hz, 1H), 7.00 (t, J = 8 Hz, 1H), 7.06 (s, 1H), 7.18 (d, J = 8 Hz, 1H), 7.27 (m, 2H), 7.53 (d, J = 8 Hz, 1H), 10.78 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 23.16, 26.06, 30.50, 31.66, 44.53, 45.04, 111.90, 113.64, 118.49, 118.73, 121.43, 126.60, 127.31, 128.79, 130.77, 131.62, 132.00, 136.66, 138.25, 154.39, 157.42. HRMS (TOF MS ES^+) calcd for $C_{24}H_{26}Cl_2N_5$ (M + H) 465.1361; Found: 465.1356.

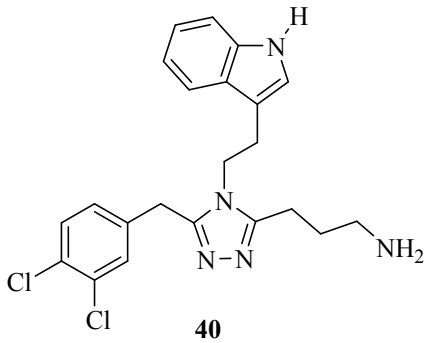
1.2.33. 3-(2-(5-(2-(1*H*-Imidazol-4-yl)ethyl)-4-phenethyl-4*H*-1,2,4-triazol-3-yl)ethyl)-1*H*-indole (**39**).



39

Thioamide **22** (1.26 g, 4.09 mmol), hydrazide **5** (3.22 g, 8.12 mmol), and mercuric acetate (1.43 g, 4.49 mmol) in THF (175 mL) was stirred under a nitrogen atmosphere for 48 h. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (70 mL), washed with $NaHSO_4$ (3 x 60 mL) and H_2O (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The trityl-protected intermediate was purified by silica gel flash chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (98:1:1) to CH_2Cl_2/MeOH/NH_4OH (84:15:1) as the eluents. The trityl-protecting group was removed by refluxing overnight in a mixture of EtOH (170 mL) and 1 N HCl (23 mL). The EtOH was evaporated and H_2O (50 mL) was added. The acidic solution was extracted with CH_2Cl_2 (3 x 60 mL), basified with 2 N NaOH, and extracted with CH_2Cl_2 (3 x 60 mL). Evaporation of the CH_2Cl_2 extracts gave the crude product which was purified by column chromatography using CH_2Cl_2 followed by gradient solvent system starting with CH_2Cl_2/CH_3OH/NH_4OH (98:1:1:1) to CH_2Cl_2/CH_3OH/NH_4OH (84:15:1) as the eluents to yield 0.280 (17%) of a yellow foam: ^{1}H NMR ($CDCl_3$) δ 2.58 (t, 4H), 2.81 (t, J = 7 Hz, 2H), 3.10 (t, J = 7 Hz, 2H), 3.19 (t, J = 8 Hz, 2H), 3.55 (t, J= 8 Hz, 2H), 6.62 (s, 1H), 6.71 (m, 2H), 6.79 (s, 1H), 7.08 (t, J = 2.4 Hz, 1H), 7.16 (m, 4H), 7.33 (d, J = 8 Hz, 1H), 7.45 (m, 2H), 8.76 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.01, 23.61, 25.20, 25.95, 36.35, 44.35, 111.53, 114.20, 118.47, 119.42, 122.11, 122.23, 127.07, 127.39, 128.75, 128.95, 134.74, 136.37, 136.51, 154.26, 154.82. HRMS (TOF MS ES$^+$) calcd for $C_{25}H_{27}N_6$ $(M + H)^+$ 411.2297; Found: 411.2292.

1.2.34. 3-(4-(2-(1*H*-Indol-3-yl)ethyl)-5-(3,4-dichlorobenzyl)-4*H*-1,2,4-triazol-3-yl)propan-1-amine **(40)**.

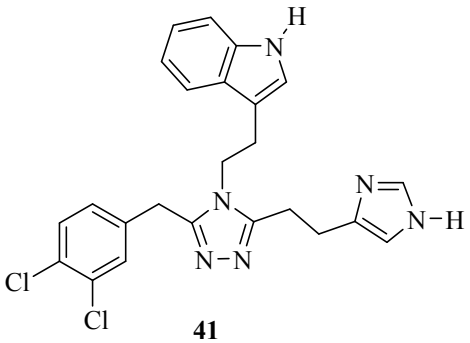


40

Thioamide **22** (1.26 g, 4.09 mmol), hydrazide **5** (3.22 g, 8.12 mmol), and mercuric acetate (1.43 g, 4.49 mmol) in THF (175 mL) was stirred under a nitrogen atmosphere for 48 h. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (70 mL), washed with $NaHSO_4$ (3 x 60 mL) and H_2O (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The trityl-protected intermediate was purified by silica gel flash chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (98:1:1) to CH_2Cl_2/MeOH/NH_4OH (84:15:1) as the eluents. The trityl-protecting group was removed by refluxing overnight in a mixture of EtOH (170 mL) and 1 N HCl (23 mL). The EtOH was evaporated and H_2O (50 mL) was added. The acidic solution was extracted with CH_2Cl_2 (3 x 60 mL), basified with 2 N NaOH, and extracted with CH_2Cl_2 (3 x 60 mL). Evaporation of the CH_2Cl_2 extracts gave the crude product which was purified by column chromatography using CH_2Cl_2 followed by gradient solvent system starting with CH_2Cl_2/CH_3OH/NH_4OH (98:1:1:1) to CH_2Cl_2/CH_3OH/NH_4OH (84:15:1) as the eluents to yield 0.280 (17%) of a yellow foam: ^{1}H NMR ($CDCl_3$) δ 2.58 (t, 4H), 2.81 (t, J = 7 Hz, 2H), 3.10 (t, J = 7 Hz, 2H), 3.55 (t, 2H), 6.62 (s, 1H), 6.71 (m, 2H), 6.79 (s, 1H), 7.08 (t, 1H), 7.16 (m, 4H), 7.33 (d, 1H), 7.45 (d, 2H), 8.76 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.01, 23.61, 25.20, 25.95, 36.35, 44.35, 111.53, 114.20, 118.47, 119.42, 122.11, 122.23, 127.07, 127.39, 128.75, 128.95,

134.74, 136.37, 136.51, 154.26, 154.82. HRMS (TOF MS ES$^+$) calcd for $C_{22}H_{24}Cl_2N_5$ $(M + H)^+$ 428.1409; Found: 428.1403.

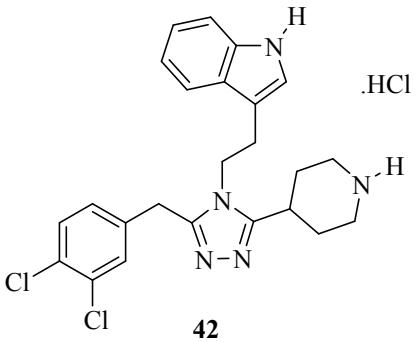
1.2.35. 3-(2-(3-(2-(1*H*-Imidazol-4-yl)ethyl)-5-(3,4-dichlorobenzyl)-4*H*-1,2,4-triazol-4-yl)ethyl)-1*H*-indole **(41)**.



41

In a manner described for **27**, thioamide **26** (1.00 g, 2.75 mmol), hydrazide **5** (1.3 g, 3.28 mmol), silver benzoate (1.26 g, 5.50 mmol), and glacial HOAc (0.51 mL, 8.25 mmol) were dissolved in CH_2Cl_2 (100 mL) and stirred for 29 h. The trityl-intermediate was subjected to silica gel chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1), and the purified intermediate was refluxed overnight in a mixture of EtOH (50 mL) and 1N HCl (50 mL). Removal of the solvents under reduced pressure followed by the normal acid-base work-up gave, after trituration with acetone, 0.75 g (59%) of a cream-colored solid: mp 248-250 ^oC; ^1H NMR (DMSO-d_6) δ 2.79 (br s, 6H), 3.79 (s, 2H), 3.96 (t, 2H), 6.69 (br s, 1H), 6.99 (m, 2H), 7.03 (m, 2H), 7.29 (m, 3H), 7.45 (s, 1H), 7.52 (d, J = 8 Hz, 1H), 10.91 (s, 1H), 11.73 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 24.86, 25.96, 26.20, 29.66, 44.05, 110.34, 112.04, 112.46, 118.43, 119.10, 121.70, 124.04, 127.33, 129.21, 129.58, 131.06, 131.22, 131.45, 135.06, 136.62, 138.26, 139.93, 152.26, 154.74. HRMS (TOF MS ES$^+$) calcd for $C_{24}H_{23}Cl_2N_6$ $(M + H)^+$ 465.1361; Found 465.1356.

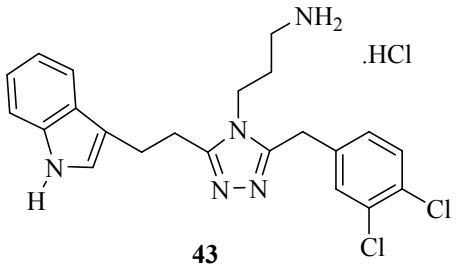
1.2.36. 3-(2-(3-(3,4-Dichlorobenzyl)-5-(piperidin-4-yl)-4*H*-1,2,4-triazol-4-yl)ethyl)-1*H*-indole hydrochloride **(42)**.



42

Following the procedure described for **27**, thioamide **26** (0.870 g, 2.39 mmol), hydrazide **7** (0.700 g, 2.88 mmol), silver benzoate (1.09 g, 4.76 mmol), and glacial HOAc (0.5 mL, 7.14 mmol) were dissolved in CH_2Cl_2 (60 mL) and stirred under a nitrogen atmosphere for 45 h. The Boc-protected intermediate was purified by silica gel flash chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1) as the eluents. Removal of the Boc-protecting group was accomplished by refluxing in a mixture of EtOH (50 mL) and 1N HCl (10 mL). An acid-base work-up gave the target triazole. The free base was dissolved in CH_2Cl_2 (5 mL) and treated with 1.25M HCl in MeOH. After standing overnight, the precipitate was filtered and dried to give 0.490 g (42%) of a cream-colored solid: mp 240-245 ^oC; ^1H NMR (DMSO-d_6) δ 1.31 (d, J = 8 Hz, 2H), 1.53 (m, 2H), 2.27 (m, 3H), 2.83 (t, J = 8 Hz, 2H), 2.96 (d, J = 12 Hz, 2H), 3.97 (s, 2H), 4.09 (t, J = 8 Hz, 2H), 6.84 (d, J = 2 Hz, 1H), 6.93 (t, J = 12 Hz, 1H), 7.05 (t, J = 12 Hz, 1H), 7.17 (dd, J = 8, 1.6 Hz, 1H), 7.31 (d, J = 12 Hz, 2H), 7.41 (d, J = 2 Hz, 1H), 7.55 (d, J = 8 Hz, 1H), 10.89 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 26.30, 29.53, 29.69, 30.90, 43.69, 44.40, 110.14, 112.06, 118.48, 119.16, 121.79, 124.29, 127.36, 129.84, 131.09, 131.42, 131.45, 136.73, 138.29, 152.18, 157.57. HRMS (TOF MS ES^+) calcd for $C_{24}H_{27}Cl_3N_5$ $(M + H)^+$ 490.1332; Found; 490.1327.

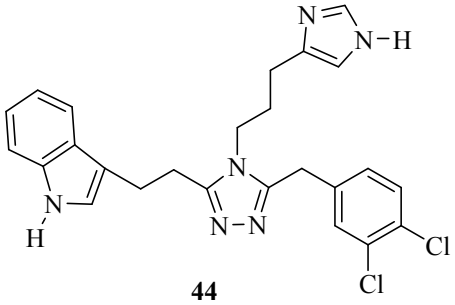
1.2.37. 3-(3-(2-(1*H*-Indol-3-yl)ethyl)-5-(3,4-dichlorobenzyl)-4*H*-1,2,4-triazol-4-yl)propan-1-amine hydrochloride **(43)**.



43

In a manner described for **27**, thioamide **24** (1.73 g, 3.43 mmol), hydrazide **8** (0.892 g, 4.07 mmol), silver benzoate (1.55 g, 6.77 mmol), and glacial HOAc (0.88 g, 15.2 mmol) in CH_2Cl_2 (100 mL) were stirred under nitrogen for 45 h. The trityl-intermediate was purified by silica gel chromatography using EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1) as the eluents. Removal of the trityl group was accomplished by refluxing in a mixture of EtOH (80 mL) and 1 N HCl (30 mL). After evaporation of the EtOH and an acid-base work-up, a hydrochloride salt was formed with 1.25 M HCl in MeOH to yield 0.360 g (23%) of **43** as a white foam: ^{1}H NMR (DMSO-d_6) δ 1.51 (p, 2H), 2.51 (t, *J* = 8 Hz, 2H), 2.98 (t, *J* = 8 Hz, 2H), 3.09 (t, *J* = 8 Hz, 2H), 3.80 (t, *J* = 8 Hz, 2H), 4.11 (s, 2H), 6.92 (t, *J* = 8 Hz, 1H), 7.02 (t, *J* = 8 Hz, 1H), 7.14 (d, *J* = 2.4 Hz, 1H), 7.19 (dd, *J* = 8, 1.6 Hz, 1H), 7.29 (d, *J* = 8 Hz, 1H), 7.45 (d, *J* = 8 Hz, 1H), 7.53 (s, 1H), 7.54 (m, 1H), 10.81 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 23.14, 25.95, 29.59, 31.63, 37.90, 111.89, 113.93, 118.75, 118.78, 121.44, 123.18, 127.47, 129.64, 129.85, 131.14, 131.27, 131.46, 136.71, 138.47, 152.42, 154.71. HRMS (TOF MS ES$^+$) calcd for $C_{22}H_{25}Cl_3N_5$ $(M + H)^+$ 464.1176; Found 464.1170.

1.2.38. 3-(2-(4-(3-(1*H*-Imidazol-4-yl)propyl)-5-(3,4-dichlorobenzyl)-4*H*-1,2,4-triazol-3-yl)ethyl)-1*H*-indole **(44)**.



44

Using the general procedure described for **27**, thioamide **23** (1.05 g, 1.89 mmol), hydrazide **8** (0.50 g, 2.28 mmol), silver benzoate (0.867 g, 3.79 mmol), and glacial HOAc (0.328 g, 5.68 mmol) in CH_2Cl_2 (100 mL) were stirred under a nitrogen atmosphere for 7 h. The trityl-protected intermediate was purified by silica gel flash chromatography eluting with EtOAc followed by EtOAc/MeOH/NH_4OH (95:4:1) as the solvents. The chromatographed product was refluxed in a mixture of EtOH (50 mL) and 1N HCl (20 mL). Removal of the solvents under reduced pressure and an acid-base work-up yielded 0.562 g (62%) of a white solid: mp 179-183 ^oC; ^1H NMR (DMSO-d_6) δ 1.62 (p, 2H), 2.34 (t, J = 8 Hz, 2H), 2.92 (t, J = 8 Hz, 2H), 3.06 (t, J = 8 Hz, 2H), 3.70 (t, J = 8 Hz, 2H), 4.04 (s, 2H), 6.72 (br s, 1H), 6.91 (t, J = 7 Hz, 1 H), 7.01 (t, J = 8 Hz, 1H), 7.09 (m, 2H), 7.28 (d, J = 12 Hz, 1H), 7.45 (m, 4H), 10.78 (s, 1H), 11.74 (b, 1H); ^{13}C NMR (DMSO-d_6) δ 23.27, 24.91, 25.91, 29.65, 30.01, 42.53, 111.86, 112.52, 113.85, 118.69, 118.79, 121.43, 123.12, 127.44, 129.53, 129.84, 131.11, 131.47, 135.22, 136.68, 138.43, 139.80, 152.34, 154.74. HRMS (TOF MS ES^+) calcd for $C_{25}H_{25}Cl_2N_6$ $(M + H)^+$ 479.1518; Found 479.1512.

1.1. *Radioligand Binding*

Competitive radioligand binding experiments were performed using Membrane Target™ Systems (Perkin-Elmer, Boston, MA) for human somatostatin sst_4 (ES-524-M400UA) and sst_{2A} (ES-521-M400UA) receptors as previously described.[11] Respective sst_4 and sst_{2A} membrane receptor preparations were prepared in assay buffer (25 mM HEPES, 10 mM $MgCl_2$, 1 mM $CaCl_2$,

0.5% BSA, pH = 7.4) at a 1:150 dilution. Binding assays were performed using ^{125}I-Tyr-SRIF 14 (Perkin-Elmer) dissolved in 1 mM HCl. For sst_{2A} the concentration of radioactivity = 0.075 nM, K_D = 0.043 nM and for sst_4 the concentration of radioactivity = 0.2625 nM, K_D = 1.05 nM. Binding assays were performed in triplicate for each concentration of ligand in a total volume of 200 µL radioligand (25 µL ligand, 25 µL radioligand, 150 µL receptors) and incubated at room temperature for 90 min using a shaking table. Binding was terminated by filtration through GF/B glass fiber filters that were presoaked in 0.5% polyethyleneimine for a minimum of 4 h. Filters were washed 9 x with 1000 µL ice cold wash buffer (50 mM Tris-HCl, pH = 7.4, 0.2% BSA). Filters were scored, transferred into plastic test tubes and counted in a gamma counter (Wizard2, Perkin-Elmer). Determination of the Ki for each compound was performed using non-linear regression with GraphPad Prism-5 software (GraphPad Software, Inc., La Jolla, CA).

1.3. *Functional Assay*

Measurement of forskolin stimulated inhibition of cAMP was performed via time-resolved fluorescence resonance energy transfer (TR-FRET) LANCE assay (AD0262, PerkinElmer Life Science, Inc., Boston MA). Recombinant Chinese hamster ovary (CHO-K1) cells expressing human somatostatin sst_4 cells (ES-524-CF, PerkinElmer Life Science, Inc., Boston MA) were thawed (37°C), resuspended in 10 mL Hanks' balanced salt solution- no phenol red (HBSS, Invitrogen, Carlsbad CA), and then centrifuged (150 x g, 5 min). Cellular pellets were resuspended in stimulation buffer containing HBSS 1x, HEPES 5 mM, Protease free BSA 0.1 % (PerkinElmer), and 3-Isobutyl-1-methylxanthine 0.5 mM (pH 7.4) and seeded in 96-well plates at 4000 cells/well. The LANCE cAMP assay was performed per manufacturer instruction with assessment of somatostatin-28 (Sigma-Aldrich Co., St. Louis, MO), NNC 26-9100, and compound **44** (0.0001–10,000 nM), against 5 µM forskolin, performed in triplicate. The cAMP calibration curve was

assessed without cells. Fluorescence signal was measured at 2.5 and 20 hrs (ex. 340 nm and em. 665 nm, 400-μs delay) via TR-FRET (FLUOstar Omega-F, BMG Labtech, Inc., Cary, NC). Data was calculated via GraphPad Prism-5 software.

1.4. *Docking protocol*

GLIDE 6.8[12,13]. The protein preparation wizard was employed to refine the protein model by assigning bond orders and adding hydrogens. Subsequently, a grid was generated using residues Asp 90 and His258, while the enclosing box was set to 19 angstroms. The van der Waals scaling factor was set at 1.0 with partial cutoff of 0.25. Docking was performed with standard precision, and flexibility with sample nitrogen inversions and ring conformations was allowed. Epik state penalties to docking score were included, while canonicalization was not permitted. Ligands docked are shown in **Figure 4**. The ligand van der Waals radii scaling factor and partial charge cutoff were set to 0.8 and 0.15, respectively. Fifteen poses per ligand were generated with post docking minimization enabled.

GOLD 5.2.2[14]. Docking experiments were also performed using GOLD 5.2.2. After protein preparation, the active site was specified using the same residues as above and a 10 Å radius. Cavity detection and forcing all H bond donors/acceptors to be treated as solvent accessible were both enabled. Fifteen poses per ligand were generated. GoldScore was the primary scoring function employed, followed by rescoring with ChemScore fitness function. Early termination was not allowed, and the use of internal ligand energy offset was selected. Search efficiency was set at 127%.

1.5. *Virtual Screening*

GLIDE 6.8. The sst_4 protein was prepared with the protein preparation wizard workflow as described in 4.4. The ligand library consisted of all molecules in **Figure 4** and 996 lead-like

decoys. The ligand van der Waals radii scaling factor and partial charge cutoff were set to 0.8 and 0.15, respectively. One pose per ligand was generated with post docking minimization enabled.

GOLD 5.2.2 Same compound library as in VS with Glide was also employed here with one pose per ligand being generated. GoldScore was the primary scoring function, followed by ChemScore rescoring. Early termination was not allowed and the use of internal ligand energy offset was selected.

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