Indian Journal of Chemistry
Vol. 58B, November 2019, pp. 1257-1272

Design, synthesis and characterization of novel paracetamol derivatives to target breast cancer

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Received 12 June 2018; accepted (revised) 16 May 2019

Most breast cancers are Estrogen Receptor-positive type. In the mammary epithelial, estrogen controls many cellular activities such as proliferation, differentiation and migration. There are two genetically distinct and functional estrogen receptors (ERs), ERα and ERβ, belonging to the superfamily of nuclear receptors for steroid/thyroid harmones. Estrogenexert its functions in different tissues by binding with its receptors, including alpha and beta (ERα and ERβ). Estrogen Receptor alpha (ERα) controls breast tissue development and progression of breast cancer. Paracetamol is one of the most widely used medicines. A recent experimental study suggests that paracetamol may have several pharmacological effects other than its well known analgesic/antipyretic properties. The docking study was performed on different paracetamol derivatives using Schrodinger 2015 (maestro 10.1) on Human Estrogen Receptor Alpha Ligand-Binding Domain (1XP6) and Endothelial nitric oxide synthase (3NLE). The *in silico* studies indicate that N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide derivatives exhibit comparable docking score and good hydrogen bond interactions at Ligand binding domain of ERα and 3NLE. Based on the docking studies, a new series of N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide derivatives have been synthesized by employing click chemistry approach. Nine compounds have been evaluated for their cytotoxicity in MCF-7 cell line and anti oxidant activity. Many of the synthesized compounds exhibit potent cytotoxic and anti oxidant activity. In particular **5c**, **5g**, and **5b** compounds show most potent cytotoxicity with IC_{50} value of 19.83, 20.57, 20.83 µg/mL respectively and **5e** and **5f** show most potent anti oxidant activity with IC_{50} value of 0.4, 0.5 µg/mL respectively.

Keywords: N-(4-((1*H*-1,2,3-Triazol-4-yl)methoxy)phenyl)acetamide, click chemistry, docking, estrogen receptor, MCF-7 cell line, anti-oxidant activity

Estrogen receptor-positive (ER+) breast cancer is the most common type of breast cancer diagnosed today. There are many established risk factors forbreast cancer, including age, genetic alterations, family history, mammographic breast density, menstrual and menopausal his-tory, radiation exposure, and life style. In particular, thehormones, estrogen and/or progesterone, are known to be capable of increasing breast cancer risk[1-3]. According to the American Cancer Society, about two out of every three cases of breast cancer are hormone receptor positive. Most of these cases are ER+ or receptive to both estrogen and progesterone. In Estrogen receptor positive breast cancer the level of Estrogen is a key factor for the initiation and progression of breast cancer[4-7]. In the mammary epithelial, estrogen controls many cellular activities such as proliferation, differentiation and migration[8,9]. There are two genetically distinct and functional estrogen receptors(ERs), ERα and ERβ, belonging to the superfamily of nuclear receptors for steroid/thyroid harmones. The structural differences between the two ERs indicate that they serve distinct actions[10]. Estrogen exert its functions in different tissues by binding with its receptors, including alpha and beta (ERα and ERβ), the former is the major one involved in breast cancer and chosen as an important target for endocrine therapy in clinic[11].

Paracetamol is a widely used over-the-counter pain medication and medication to reduce fever[12]. Paracetamol is used in the management of more severe pain such as post surgical and cancer pain in combination with opioid analgesics. In addition to well known use pain relief and fever reduction, recent laboratory and pre-clinical studies have demonstrated

that Paracetamol may also have beneficial effects on blood glucose levels, skeletal muscle function, and potential use as cardioprotective and neuroprotective agent. These effects may be derived from the ability of Paracetamol to function as an antioxidant. Thus Paracetamol continues to attract considerable scientific attention because of its association with a variety of biological activities.

1,2,3-Triazole moieties are attractive connecting units because they are stable to metabolic degradation and capable of hydrogen bonding, which can be favorable in the binding of biomolecular targets and can improve the solubility[13,14]. The importance of triazolic compounds in medicinal chemistry is undeniable.

An emerging strategy within medicinal chemistry and drug discovery is the combination of two distinct pharmacophores into a single molecule, well documented as molecular hybridization (MH). In the present study, we made an attempt to combine Paracetamol and Triazole moieties carrying groups at selected positions by employing hybridization approach to target Human Estrogen Receptor Alpha Ligand-Binding Domain.

The docking study was performed on different Paracetamol derivatives using Schrodinger 2015 (maestro 10.1) on Human Estrogen Receptor Alpha Ligand-Binding Domain (1XP6) and Endothelial nitric oxide synthase (3NLE). The insilico studies indicated that N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide derivatives exhibited comparable docking score and good hydrogen bond interactions. Based on the docking studies a new series of N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide derivatives were synthesized by employing Click-Chemistry Approach with an aim to obtain possible novel breast cancer agents.

Experimental Section

Chemistry

All chemicals and dry solvents were purchased from the local manufacturers and S.D Fine Chem. Ltd, Mumbai, India. All the chemicals used in the synthesis were obtained from standard commercial sources. All Reactions were monitored by thin layer chromatography (TLC) carried out on E. Merck silica gel plates (60 F_{254}) with UV light, iodine as probing agents. Column chromatography separation was performed using Avra Synthesis Pvt. Ltd. Silica gel 60, 0.140-0.25 mm (60-120 mesh) using combination of Ethyl acetate and Hexane. Melting points were determined on an Digital melting point apparatus (Jain Scientific glass works) by open capillary method and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity 400 or Varian Inova 500 or Bruker Avance 300 MHz. Chemical shifts are relative to TMS as an internal standard. Mass spectra recorded on Agilent LC/MSD trap SL 1100 series spectrometer with a 70 eV (ESI probe). Infrared (IR) spectra were recorded a Thermo nicolet Nexus 670 FT-IR spectrometer, Perkin-Elmer Infrared-683 or 1310 with NaCl optics. The names of all compounds given in the experimental section were taken from Chemdraw Ultra, Version 8.0. All the reactions were carried out in dried glassware under an atmosphere of nitrogen.

Aryl alcohols, 1a-i

Required quantities of Aryl Aldehyde is dissolved in Methanol (1 g=10 mL) then Sodium borohydride (2eqt) was added and stirred at 0°C. After completion of reaction (monitored by TLC), excess Sodium borohydride quenched by adding cold water or Ammonium chloride solution. Evaporate the methanol and then work up with ethyl acetate and water. The organic phase was dried with sodium sulphate and concentrated and then The residue was purified by column chromatography to afford pure compound.

Aryl bromides, 2a-i

Required quantities of Aryl Alcohols **1a-i** were dissolved in Ether (1 g=10 mL) then Phosphorus tribromide (0.5 eqt) was added and stirred at 0°c. After completion of reaction (monitored by TLC), excess Phosphorus tribromide quenched by adding sodium carbonate solution. And then work up with ethyl acetate and water. The organic phase was dried with sodium sulphate and concentrated and then The residue was purified by column chromatography to afford pure compounds **2a-i**.

Aryl Azides, 3a-i

To a solution of Aryl Bromides **2a-i** (1 g) in Dichloromethane (10 mL) and water (5 mL) was added Sodium azide (2 eqt) and catalytic amount of TBAB (Tetra-n-butylammonium bromide). The resulting mixture was stirred overnight. After completion of reaction (monitored by TLC), then work up with equal amount of DCM and water. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude material was purified by column chromatography[15].

N-(4-(Prop-2-ynyloxy)phenyl)acetamide, 4

Required quantities of Paracetamol dissolved in Dimethyl formamide (1 g=10 mL) then pottasium carbonate (1.2eqt) and propargyl bromide (1 eqt) was added and stirred for 24 h at RT. After completion of reaction (monitored by TLC), diluted slowly by adding crushed ice, citric acid and stirred again for 1 hr. The crystals were filtered off. The solid was diluted with ethyl acetate (40 mL) and washed with water (20 mL).The organic phase was dried with sodium sulphate and concentrated and then The residue was purified by column chromatography to afford pure compound.

N-(4-((1*H*-1,2,3-Triazol-4-yl)methoxy)phenyl) acetamide, 5a-i

0.2 g of N-(4-(prop-2-ynyloxy)phenyl) acetamide (4) dissolved in Tetrahydrofuran (4 mL), then add Aryl Azides **3a-i** (1.1eqt), water (3 mL), copper sulphate, Na Ascorbate (catalytic amounts) stirred for 24 hr at rt. After completion of reaction (monitored by TLC), then work up with equal amount of Ethyl acetate and water. The organic phase was dried with sodium sulphate and concentrated. The residue was purified by column chromatography to afford pure compounds **5a-i**. Physical characterization data of Paracetamol derivatives **5a-i** are tabulated in Table I.

N-(4-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy) phenyl) acetamide, 5a: Yield 98%. m.p.130-133°C. IR (KBr): 3297, 3202, 2872, 1678, 1598, 1232, 1015, 839, 722, 691 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.66 (1H, s, N*H*-CO-CH_3), 7.53 (1H, s, H-2), 7.37 (5H, m, H-6, H-5′, (H-3″,4″,5″ Benzyl), 7.27 (2H, d, *J* = 5.7 HZ, H-2″, H-6″,Benzyl), 6.86 (2H, d, *J* = 9.0 HZ, H-3, H-5), 5.52 (2H, s, O-CH_2), 5.12 (2H, s, CH_2-C_6H_5), 2.13 (3H, s, NH-CO-CH_3); ^{13}C NMR ($CDCl_3$): δ 168.4 (C, NH-*C*O-CH_3), 154.8 (C, C-4), 144.4 (C, C-4′), 134.3 (C, C-1″ Bz), 131.5 (C, C-1), 129.1 (CH, C-2″, C-6″ Bz), 128.7 (CH, C-3″, C-5″ Bz), 128.0 (CH, C-4″ Bz), 122.6 (CH, C-2, C-6), 121.8 (CH, C-5′), 115.0 (CH, C-3, C-5), 62.1 (CH_2, O-CH_2), 54.2 (CH_2, CH_2-C_6H_5), 24.2 (CH_3, NH-CO-CH_3); MS: *m/z* 323.25 $(MH)^+C_{18}H_{18}N_4O_2$.

N-(4-((1-(4-Chlorobenzoyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide, 5b: Yield 92%. m.p.207°C; IR (KBr): 3259, 3053, 2931, 1699, 1679, 1508, 1301, 1233, 1093, 841, 748 cm^{-1};^{1}H NMR (DMSO-d_6): δ 9.82 (1H, s, N*H*-CO-CH_3), 8.18 (1H, s, H-6″Benzoyl), 8.07 (2H, d, *J* = 7.0 HZ, H-6, (H-2″Benzoyl)), 7.60 (2H, t, *J* = 7.5 HZ, H-2, H-5″Benzoyl), 7.49 (2H, d, *J* = 9.0 HZ, H-5′(H-3″Benzoyl)), 6.99 (2H, d, *J* = 9.0 HZ, H-3, H-5), 6.19 (2H, s, O-CH_2), 5.15 (2H, s, CH_2-CO), 2.00 (3H, s, NH-CO-CH_3); ^{13}C NMR (DMSO-d_6): δ 191.2 (C, *C*O-CH_2), 167.8 (C, NH*C*OCH_3), 153.6 (C, C-4), 142.7 (C, C-4′), 139.1 (C, C-4″ Benzoyl), 137.7 (C, C-1″ Benzoyl), 132.7 (C, C-1), 130.0 (CH, C-2″, C-6″ Benzoyl), 129.1 (CH, C-3″, C-5″ Benzoyl), 126.1 (CH, C-2, C-6), 120.4 (CH, C-5′), 114.7 (CH, C-3, C-5), 61.1 (CH_2, O-CH_2), 55.8 (CH_2, CH_2-CO), 23.7 (CH_3, NHCOCH_3); MS: *m/z* 385 $(MH)^+C_{19}H_{17}ClN_4O_3$.

N-(4-((1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide, 5c: Yield 98%. m.p.163-165°C. IR (KBr): 3290, 3091, 2930, 1615, 1410, 1223, 1176, 1130, 1013, 851 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.81 (1H, s, N*H*-CO-CH_3), 8.25 (1H, s, H-6), 7.46 (2H, d, *J* = 9.0 HZ, H-2, H-5′), 7.37-7.39 (2H, m, H-2″, H-6″,Benzyl), 7.19 (2H, t, *J* = 8.8 HZ, H-3″, H-5″, Benzyl), 6.94 (2H, d, *J* = 9.0 HZ, H-3, H-5), 5.58 (2H, s, O-CH_2), 5.07 (2H, s, CH_2-C_6H_5), 2.00 (3H, s, NH-CO-CH_3); ^{13}C NMR (DMSO-d_6): δ 167.9 (C, NH-*C*O-CH_3), 160.9 (C, C-4″ Bz),153.7 (C, C-4), 143.1 (C, C-4′), 132.8 (C, C-1″ Bz), 132.2 (C, C-1), 130.3 (CH, C-2″ Bz), 130.2 (CH, C-6″ Bz), 124.5 (CH, C-2, C-6), 120.5 (CH, C-5′), 115.7 (CH, C-3″ Bz), 115.5 (CH, C-5″ Bz), 114.7 (CH, C-3, C-5) 61.2 (CH_2, O-CH_2), 52.0 (CH_2, CH_2-C_6H_5), 23.7 (CH_3, NHCOCH_3); MS: *m/z* 341 $(MH)^+$ $C_{18}H_{17}FN_4O_2$.

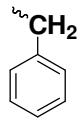
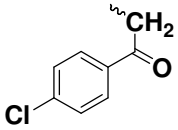
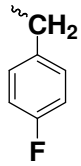
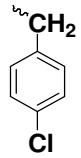
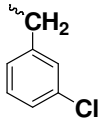
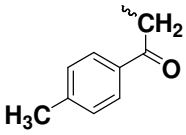
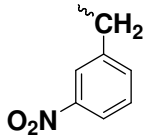
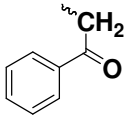
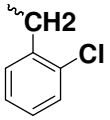
N-(4-((1-(4-Chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)acetamide, 5d: Yield 97%. m.p.187-189°C. IR (KBr): 3445, 2930, 2874, 1663, 1513, 1176, 1013, 825, 669 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.98 (1H, s, N*H*-CO-CH_3), 8.31 (2H, d, *J* = 14.3 HZ, H-2, H-6), 7.24-7.51 (5H, m, H-5′, (H-2″, H-6″, H-3″, H-5″, Benzyl), 6.93 (2H, d, *J* = 8.8 HZ, H-3, H-5), 5.61 (2H, s, O-CH_2), 5.07 (2H, s, CH_2-C_6H_5), 2.00 (3H, s, NH-CO-CH_3); ^{13}C NMR (DMSO-d_6): δ 167.8 (C, NH-*C*O-CH_3), 153.6 (C, C-4), 143.1 (C, C-4′), 134.9 (C, C-1″ Bz), 132.9 (C, C-4″ Bz), 132.8 (C, C-1), 130.6 (CH, C-2″, C-6″ Bz), 129.8 (CH, C-3″, C-5″ Bz), 128.7 (CH, C-2, C-6), 120.4 (CH, C-5′), 114.6 (CH, C-3, C-5), 79.1 (CH_2, O-CH_2), 51.9 (CH_2, CH_2-C_6H_5), 23.7 (CH_3, NH-CO-CH_3);MS: *m/z* 357$(MH)^+$ $C_{18}H_{17}ClN_4O_2$.

N-(4-((1-(3-Chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide, 5e: Yield 98%. m.p.153-155°C; IR (KBr): 3421, 2925, 2855, 1662, 1553, 1223, 1171, 1021, 866, 744, 672 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.80 (1H, s, N*H*-CO-CH_3), 8.31 (1H, s,

H-6), 7.39-7.49 (5H, m, H-2, H-5′, (H-2″, H-4″, H-5″, Benzyl)), 7.27 (1H, s, H-6″ Benzyl), 6.95 (2H, d, J = 8.8 HZ, H-3, H-5), 5.63 (2H, s, O-CH_2), 5.09 (2H, s, CH_2-C_6H_5), 2.00 (3H, s, NH-CO-CH_3); ^{13}C NMR (DMSO-d_6): δ 167.4 (C, NH-*C*O-CH_3), 153.3 (C, C-4), 142.8 (C, C-4′), 138.0 (C, C-1″ Bz), 132.9 (C, C-3″ Bz), 132.5 (C, C-1), 130.3 (CH, C-5″ Bz), 127.8 (CH, C-2″ Bz), 127.4 (CH, C-6″ Bz), 126.3 (CH, C-4″ Bz), 124.4 (CH, C-2, C-6), 120.1 (CH, C-5′) 114.4 (CH, C-3, C-5), 60.9 (CH_2, O-CH_2), 51.7 (CH_2, CH_2-C_6H_5), 23.4 (CH_3, NH-CO-CH_3); MS: *m/z* 357 $(MH)^+$ $C_{18}H_{17}ClN_4O_2$.

N-(4-((1-(4-Methylbenzoyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide, 5f: Yield 90%.

Table I — Physical characterization data of Paracetamol derivatives

Compd	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (°C)	Yield (%)
5a		$C_{18}H_{18}N_4O_2$	322.14	130-133	98
5b		$C_{19}H_{17}ClN_4O_3$	384.1	207	92
5c		$C_{18}H_{17}FN_4O_2$	340.13	163-165	98
5d		$C_{18}H_{17}ClN_4O_2$	356.1	187-189	97
5e		$C_{18}H_{17}ClN_4O_2$	356.1	153-155	98
5f		$C_{20}H_{20}N_4O_3$	364.14	209	90
5g		$C_{18}H_{17}N_5O_4$	367.13	162-164	97
5h		$C_{19}H_{18}N_4O_3$	350.14	186-188	90
5i		$C_{18}H_{17}ClN_4O_2$	356.1	176-178	98

m.p.209°C; IR (KBr): 3260, 2935, 2871, 1683, 1602, 1506, 1299, 1233, 1178, 840 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.82 (1H, s, N*H*-CO-CH_3), 8.30 (1H, s, H-6″ Benzoyl), 8.17 (1H, s, H-2″ Benzoyl), 7.97 (2H, d, *J* = 6.9 HZ, H-2, H-6), 7.44 (3H, dd, *J* = 7.7, 8.8 HZ, H-5′, (H-3″, H-5″ Benzoyl), 6.98 (2H, d, *J* = 9.0 HZ, H-3, H-5), 6.16 (2H, s, O-CH_2), 5.14 (2H, s, CH_2-CO), 2.41 (3H, s, CH_3-Benzoyl) 2.00 (3H, s, NH-CO-CH_3); ^{13}C NMR (DMSO-d_6): δ 191.5 (C, *C*O-CH_2), 167.7 (C, NH*C*OCH_3), 153.7 (C, C-4), 144.8 (C, C-4″ Benzoyl), 142.6 (C, C-4′), 132.7 (C, C-1″ Benzoyl), 131.5 (C, C-1), 129.4 (CH, C-3″, C-5″ Benzoyl), 128.2 (CH, C-2″, C-6″ Benzoyl), 126.1 (CH, C-2, C-6), 120.4 (CH, C-5′), 114.6 (CH, C-3, C-5), 79.0 (CH_2, O-CH_2), 55.6 (CH_2, CH_2-CO), 23.7 (CH_3, CH_3-Benzoyl), 21.2 (CH_3, NHCOCH_3); MS: *m/z* 365 $(MH)^+C_{20}H_{20}N_4O_3$.

N-(4-((1-(3-Nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide, 5g: Yield 97%. m.p.162-164°C; IR (KBr): 3289, 3091, 2931, 1662, 1412, 1560, 1349, 1246, 1062, 1014, 905, 780 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.80 (1H, s, N*H*-CO-CH_3), 8.35 (1H, s, H-4″Benzyl), 8.20 (2H, t, *J* = 8.1 HZ, H-6, (H-2″Benzyl), 7.76 (1H, d, *J* = 7.7 HZ, H-2), 7.67 (1H, t, *J* = 7.9 HZ H-6″Benzyl), 7.47 (2H, d, *J* = 9.0 HZ, H-5′, (H-5″Benzyl), 6.94 (2H, d, *J* = 8.9 HZ, H-3, H-5), 5.78 (2H, s, O-CH_2), 5.09 (2H, s, CH_2-C_6H_5), 2.00 (3H, s, NH-CO-CH_3); ^{13}C NMR (DMSO-d_6): δ 167.6 (C, NH-*C*O-CH_3), 153.4 (C, C-4), 147.6 (C, C-3″ Bz), 143.0 (C, C-4′), 137.7 (C, C-1″ Bz), 134.4 (CH, C-6″ Bz), 132.6 (C, C-1), 130.1 (CH, C-5″ Bz), 124.6 (CH, C-2″ Bz), 122.9 (CH, C-2, C-6), 122.5 (CH, C-5″), 120.2 (CH, C-4″ Bz), 114.5 (CH, C-3, C-5), 60.9 (CH_2, O-CH_2), 51.5 (CH_2, CH_2-C_6H_5), 23.5 (CH_3, NH-CO-CH_3); MS: *m/z* 368 $(MH)^+$ $C_{18}H_{17}N_5O_4$.

N-(4-((1-Benzoyl-1*H*-1,2,3-triazol-4-yl)methoxy) phenyl)acetamide, 5h: Yield 90%. m.p.186-188°C; IR (KBr): 3288, 3049, 2989, 1698, 1679, 1410, 1301, 1178, 1017 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.82 (1H, s, N*H*-CO-CH_3), 8.18 (1H, s, H-6″ Benzoyl), 8.07 (2H, d, *J* = 7.0 HZ, H-6 (H-2″ Benzoyl)), 7.73 (1H, t, *J* = 7.4 HZ, H-2), 7.60 (2H, t, *J* = 7.5 HZ, H-4″, H-5″Benzoyl), 7.49 (2H, d, *J* = 9.0 HZ, H-5′, (H-3″Benzoyl), 6.99 (2H, d, *J* = 9.0 HZ, H-3, H-5), 6.19 (2H, s, O-CH_2), 5.15 (2H, s, CH_2-CO), 2.00 (3H, s, NH-CO-CH_3); ^{13}C NMR (DMSO-d_6): δ 191.7 (C, *C*O-CH_2), 167.5 (C, NH*C*OCH_3), 153.4 (C, C-4), 142.3 (C, C-4′), 133.8 (C, C-1″ Benzoyl), 133.6 (CH, C-4″ Benzoyl), 132.3 (C, C-1), 128.5 (CH, C-2″, C-6″ Benzoyl), 127.7 (CH, C-3″, C-5″ Benzoyl), 125.8 (CH, C-2, C-6), 120.1 (CH, C-5′), 114.3 (CH, C-3, C-5), 60.7 (CH_2, O-CH_2), 55.4 (CH_2, CH_2-CO), 23.3 (CH_3, NHCOCH_3); MS: *m/z* 351 $(MH)^+C_{19}H_{18}N_4O_3$.

N-(4-((1-(2-Chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)acetamide, 5i: Yield 98%. m.p.176-178°C; IR (KBr): 3135, 3088, 2925, 1662, 1514, 1171, 1316, 1021, 744, 672 cm^{-1}; 1H NMR (DMSO-d_6): δ 9.87 (1H, s, NH-CO-CH_3), 8.16 (1H, s, H-6), 7.24-7.47 (6H, m, (H-3″, H-4″, H-5″, H-6″Benzyl), H-5′, H-2), 6.91 (2H, d, J = 8.6 HZ, H-3, H-5), 5.64 (2H, s, O-CH_2), 5.05 (2H, s, CH_2-C_6H_5), 1.98 (3H, s, NH-CO-CH_3); 13C NMR (DMSO-d_6): δ 169.6 (C, NH-CO-CH_3), 154.4 (C, C-4), 146.6 (CH, C-5′), 133.4 (C, C-1″ Bz), 133.3 (C, C-2″ Bz), 132.9 (C, C-1), 131.5 (CH, C-6″ Bz), 131.1 (CH, C-3″ Bz), 130.3 (CH, C-4″ Bz), 128.3 (CH, C-5″ Bz), 125.8 (CH, C-2, C-6), 121.7 (CH, C-5′) 115.5 (CH, C-3, C-5), 61.6 (CH_2, O-CH_2), 51.4 (CH_2, CH_2-C_6H_5), 24.1 (CH_3, NH-CO-CH_3); MS: *m/z* 355 (MH)-$C_{18}H_{17}ClN_4O_2$.

Docking studies

Docking studies was performed by using Schrodinger 2015 (maestro 10.1) version software on HP Compaq 6200 Pro MT PC workstation (Intel(R) Core(TM) i7 CPU 2600 @ 3.40 GHz; 8 GB Ram, 500 GB Hard disk). Human Estrogen Receptor Alpha Ligand-Binding Domain (PDB Code: 1XP6) and Endothelial nitric oxide synthase (3NLE) were selected as the Targets. The typical structure file from the protein data bank (PDB) was not suitable for immediate use in molecular modelling calculations. A typical PDB structure file consists only of heavy atoms and may include a co-crystallized ligand, water molecules, metal ions, and cofactors. In a few PDB files, the interatomic distances in the backbone differ substantially from standard values, causing PDB reading functionality to miss some connectivity's and break the molecules in multiple chains. Some PDB structures were multimeric, and may need to be reduced to a single unit. Schrodinger had therefore assembled a set of tools to prepare proteins in a form that was suitable for modelling calculations. The Protein Preparation allows to download a protein from its raw state, (which may be having missing hydrogen atoms and incorrect bond order assignments, charge states, or orientations of various groups) and to convert to a state in which it was properly prepared for calculations. SBDD involves five steps: Protein preparation, Ligand preparation, Grid generation, Ligand docking and Scoring.

Biological Activity

Breast Cancer Activity MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using MEM and DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different concentrations of test drug were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured at 540 nm by using a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line[16].

Anti-oxidant activity

The free radical scavenging activity of all the samples was evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method by Shen *et al*. Brieflyan 0.1 mM solution of DPPH in ethanol was prepared and 1 mL of this solution was added to 3 mL of the solution of all samples in ethanol at different concentration (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity[17].

Results and Discussion

Chemistry

The general synthetic scheme of the the target compounds is described in Figure 1. The starting aryl alcohols **1a-i** were prepared by reaction of the selected Aromatic aldehydes with sodium borohydride at 0°C.The starting Aryl alcohols **1a**-i were then reacted with phosphorus tribromide at 0°C to yield aryl bromides **2a-i**. Aryl bromides **2a-i** were then reacted with sodium azide in the presence of TBAB (Tetra-*n*-butylammonium bromide) to yield aryl azides **3a-i**.

N-(4-(Prop-2-ynyloxy)phenyl)acetamide **4** was prepared by reaction of Paracetamol with propargyl bromide in the presence of potassium carbonate. The target N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide **5a-i** derivatives were synthesized by the reaction of N-(4-(prop-2-ynyloxy)phenyl)acetamide **4** with aryl azides **3a-i** in the presence of copper sulphate and sodium ascorbate to give the target N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide **5a-i** derivatives in excellent yields. The azide-alkyne cycloaddition (Click Chemistry) was introduced by K. B. Sharpless in 2001. Click reactions products are high yielding, are stereospecific, simple to perform, and can be conducted in easily removable or benign solvents.

All the Paracetamol derivatives **5a-i** exhibited characteristic absorption bands in the IR spectra (cm^{-1}) *i.e*. 3297.30 (N-H Amide), 3202.17 (C-H Aromatic), 2872.42 (C-H Aliphatic), 1678.65 (C=O Amide), 1598.33 (C=C Aromatic), 1232.63 (C-O-C), 1015.59 (C-C Aliphatic) and at other regions of the spectrum depending upon the specific substituents present in each compound. The ^{1}H NMR spectra of the Paracetamol derivatives revealed the characteristic protons in between δ 2.00 and 9.98. The ^{13}C NMR spectra of the Paracetamol derivatives exhibited the characteristic peaks of the carbonyl carbon in between δ 165-170, apart from the peaks corresponding to the other carbons. The mass spectra obtained by positive mode electron spray ionization ionization method revealed the $[M+H]^+$ions and M+Na ions.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **5a** showed the characteristic signals in between δ 2.13 and 7.66. The singlets at δ 2.13, 5.12, 5.52, 7.53, 7.66 indicates the presence of Methyl, Benzylic, Methoxy, Triazolic, NH protons respectively. Doublets at δ 6.86, 7.27, 7.37 indicates the presence of Aromatic protons. ^{13}C NMR spectrum showed Methyl, Benzylic, Methoxy, Aromatic, Triazolic, Amide Carbon signals at δ 24.21, 54.22, 62.17, 115.01, 122.68, 128.78, 129.10, 131.58, 134.32, 144.42, 154.83, 168.42.

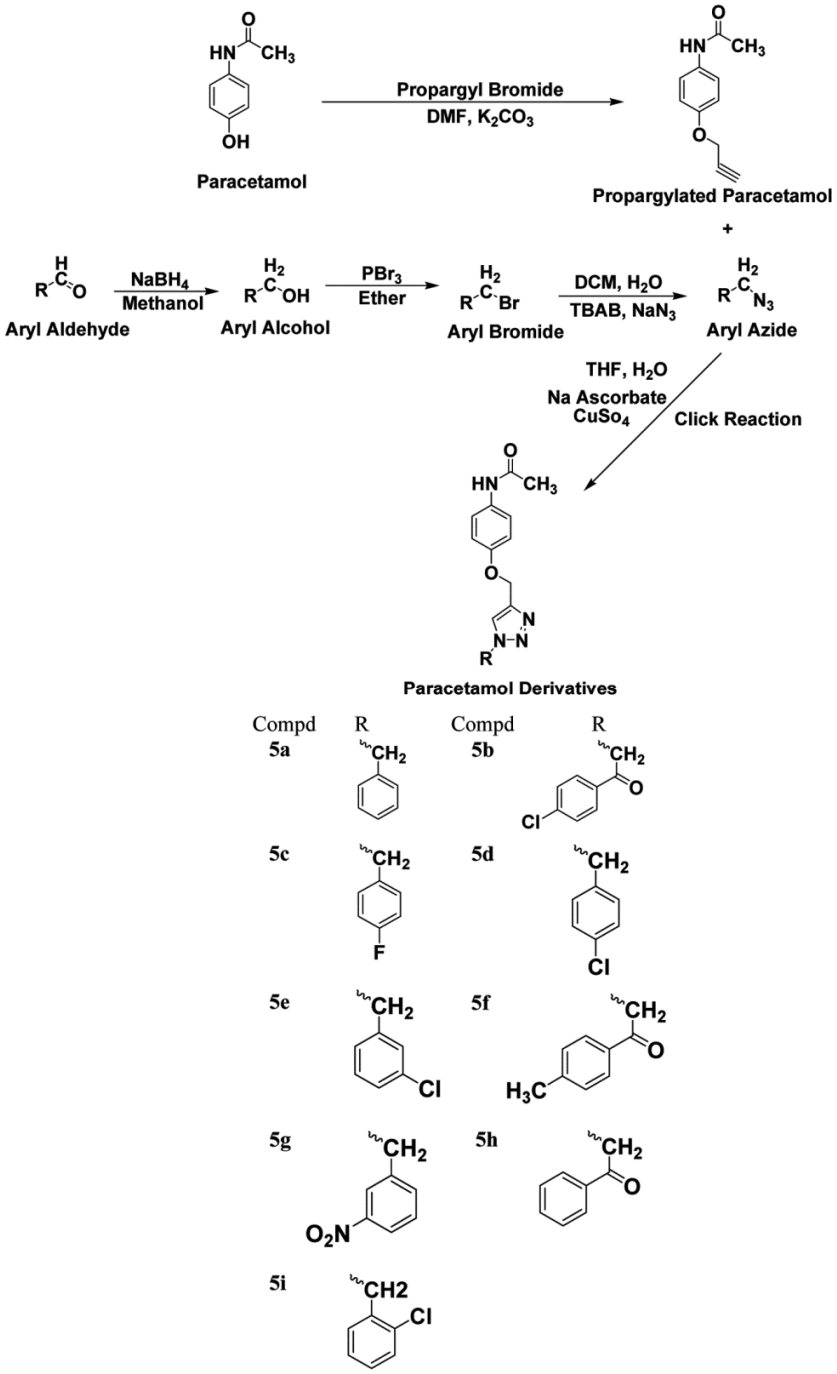


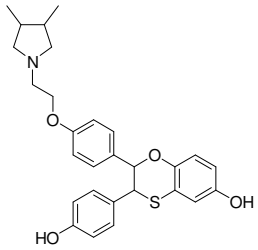
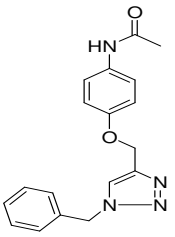
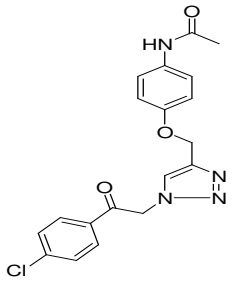
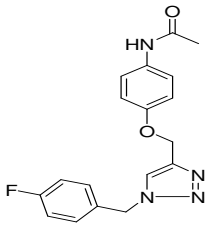
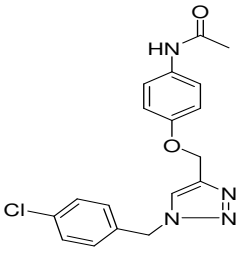
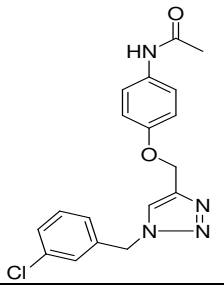
Figure 1 — Synthetic scheme of N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide derivatives

Docking

Cytotoxic activity

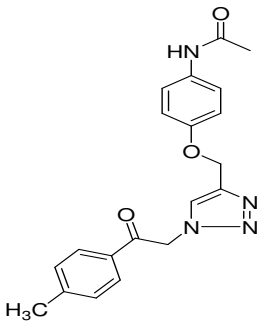
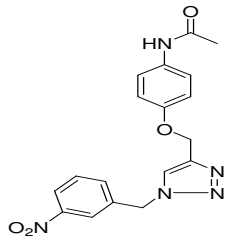
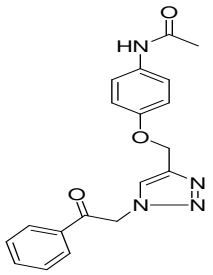
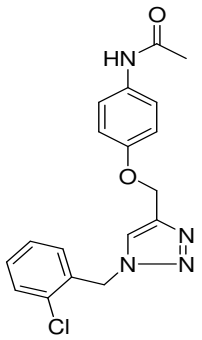
XP docking studies showed that compounds have good binding ability with Human Estrogen Receptor Alpha Ligand-Binding Domain (PDB ID: 1XP6) (Table II). All the synthesized compounds was found to have good docking score and also showing good Hydrogen bonding interaction with Amino acids present in the active site of 1XP6 (Table III). Among the synthesized compounds **5b** and **5i** was found to have more docking score *i.e.*-10.021, -9.931 respectively and showing strong Hydrogen bond

Table II — Extra precision (XP) docking results of Schrodinger:GScore, H-Bond, Glide energy values of docked ligands.1XP6 (Human Estrogen Receptor Alpha Ligand-Binding Domain In Complex With Compound 16) — (*Contd.*)

Compd	Structure	GScore	H-Bond	Glide energy (Kcal/mol)
		-14.246	-2.155	-75.647
Existing ligand				
5a		-8.264	-0.578	-46.342
5b		-10.021	-1.317	-49.14
5c		-9.582	-0.996	-37.896
5d		-8.079	-0.322	-47.482
5e		-8.478	0	-50.591

(*Contd.*)

Table II — Extra precision (XP) docking results of Schrodinger:GScore, H-Bond, Glide energy values of docked ligands.1XP6 (Human Estrogen Receptor Alpha Ligand-Binding Domain In Complex With Compound 16) — (*Contd.*)

Compd	Structure	GScore	H-Bond	Glide energy (Kcal/mol)
5f		-8.652	-0.311	-50.867
5g		-7.587	-1.306	-52.257
5h		-9.431	-0.025	-52.195
5i		-9.931	-0.213	-52.312

interaction with LYS 531, ARG 394 (Figure 2 and Figure 3). Among the synthesized compounds **5b**, **5c** and **5g** was found to have strong Hydrogen bond interaction with LYS 531, ARG 394, TYR 526, LEU 536 (Figure 4 and Figure 5).Remaining compounds in this series was found to have good docking score and showing Hydrogen bond interactions with LYS 531, TYR 526, LEU 536, ARG 394, CYS 531. Table II shows the G Score, H-Bond, Glide energy values of docked ligands and Hydrophilic and Hydrophobic Interactions.

Table III — Extra precision (XP) docking results of Schrodinger: Hydrophilic and HydrophobicInteractions of Compounds with Amino acids of 1XP6.

S.No	Compd	Interacting residues
1	**5a**	LYS 531, PHE 404
2	**5b**	LYS 531, ARG 394
3	**5c**	LYS 531
4	**5d**	LYS 531, ARG 394
5	**5e**	–
6	**5f**	CYS 531, PHE 404
7	**5g**	TYR 526, LEU 536
8	**5h**	LYS 531
9	**5i**	LYS 531, PHE 404

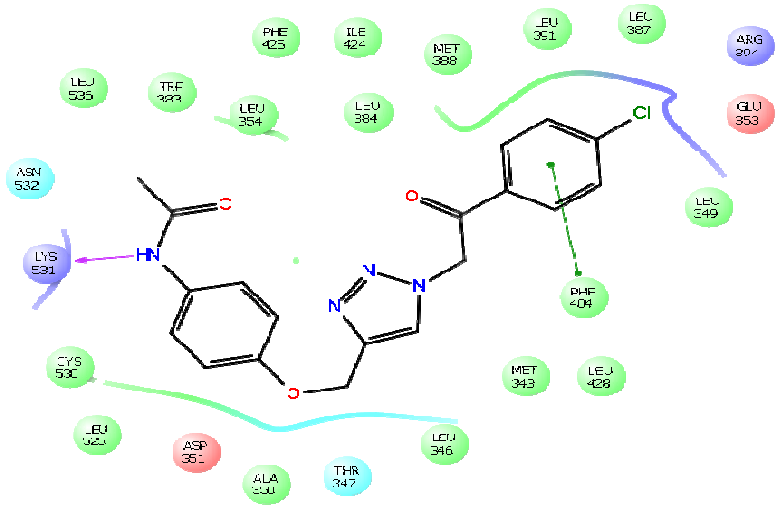


Figure 2 — Interactions (Hydrophobic, Hydrophilic) of **5b** with Amino acids present in the active site of 1XP6.

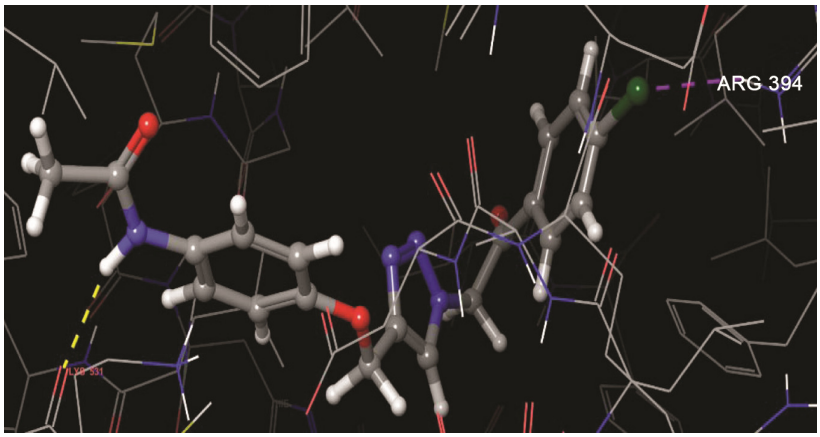


Figure 3 — H-Bonding interactions of **5b** with Amino acids present in the active site of 1XP6.

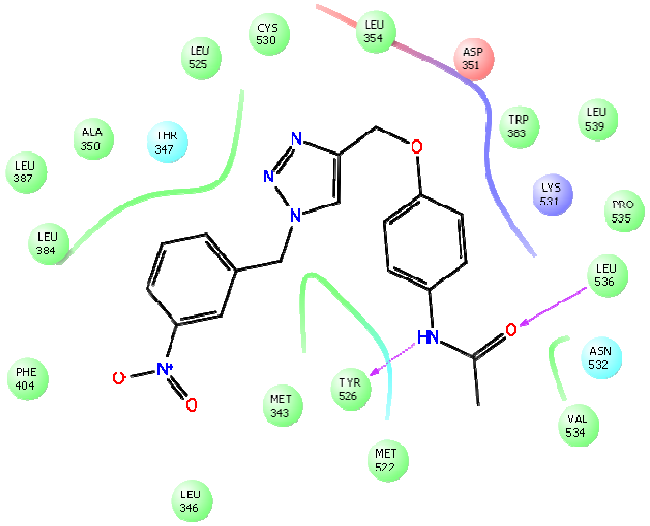


Figure 4 — Interactions (Hydrophobic, Hydrophilic) of **5g** with Amino acids present in the active site of 1XP6.

Anti-oxidant activity

XP docking studies showed that compounds have good binding ability with Endothelial nitric oxide synthase (PDB ID:3NLE) (Table IV). All synthesized compounds was found to have more docking score than Existing ligand and also showing good Hydrogen

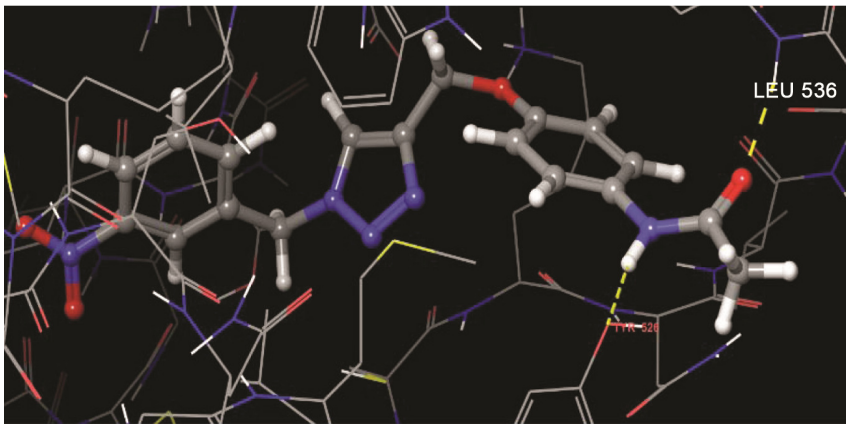
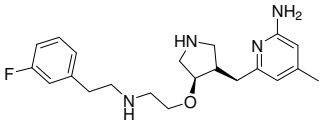
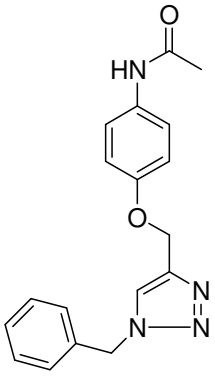
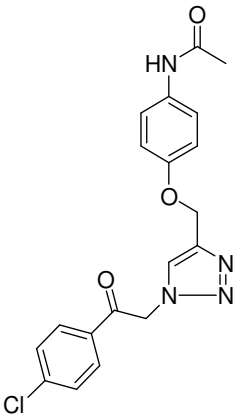


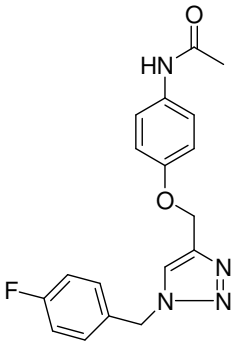
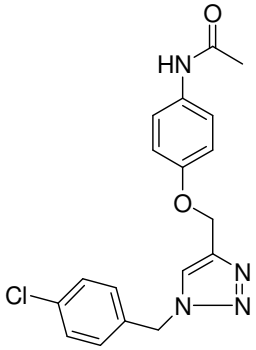
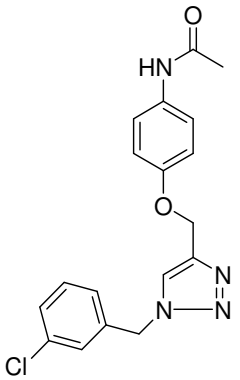
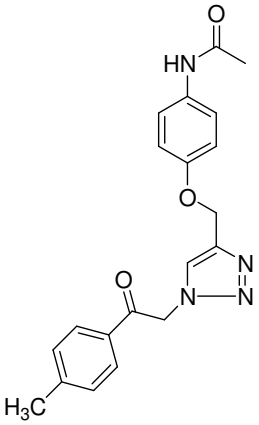
Figure 5 — H-Bonding interactions of **5g** with Amino acids present in the active site of 1XP6

Table IV — Extra precision (XP) docking results of Schrodinger:G Score, H-Bond, Glide energy values of docked ligands. 3NLE (Endothelial nitric oxide synthase) — (*Contd.*)

Compd	Structure	GScore	H-Bond	Glide energy (Kcal/mol)
Existing ligand		-6.537	0	-29.457
5a		-7.569	-0.353	-42.108
5b		-7.542	-0.7	-41.138

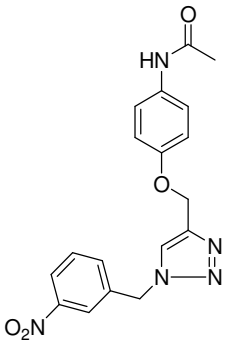
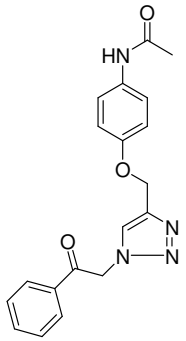
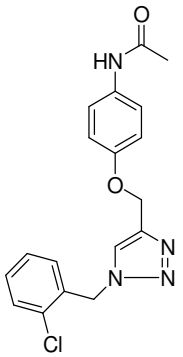
(*Contd.*)

Table IV — Extra precision (XP) docking results of Schrodinger:G Score, H-Bond, Glide energy values of docked ligands. 3NLE (Endothelial nitric oxide synthase) — (*Contd.*)

Compd	Structure	GScore	H-Bond	Glide energy (Kcal/mol)
5c		-7.832	-0.24	-39.97
5d		-7.356	0	-37.418
5e		-7.998	-0.243	-42.885
5f		-8.812	-0.026	-48.546

(*Contd.*)

Table IV — Extra precision (XP) docking results of Schrodinger:G Score, H-Bond, Glide energy values of docked ligands. 3NLE (Endothelial nitric oxide synthase).

Compd	Structure	GScore	H-Bond	Glide energy (Kcal/mol)
5g		-7.62	0	-43.318
5h		-7.215	-0.286	-42.719
5i		-7.615	0	-47.26

bonding interaction with Amino acids present in the active site of 3NLE (Figure 6, Figure 7 and Table V). In Paracetamol derivatives series (**5a** to **5i**) **5e** and **5f** was found to have more docking score *i.e.* −7.998, −8.812 respectively and showing strong Hydrogen bond interaction with TRP 358. Remaining compounds in this series was found to have good docking score and showing Hydrogen bond interactions with TYR 477, ASN 468, PHE 475.

Biological activity

MTT Assay

Nine newly synthesized compounds **5a-i** were screened for *in vitro* cytotoxic activity against human tumor cell line MCF-7 (Breast cancer cell line). The results are shown in Table VI. *In vitro* cytotoxicity results showed that all the Paracetamol derivatives showed good Cytotoxic activity. Among the compounds tested, **5c** with 4"-fluoro benzylmoiety, **5g** with 3"-nitro benzylmoiety and **5b** with 4"-chloro benzoyl moietywere found to be the most potent Compounds and having a IC_{50} value of 19.83 µg/mL,20.57 µg/mL and20.83 µg/mL respectively. This was supported by Docking studies. These Three compounds were found to have more docking score and showing strong Hydrogen bond interaction with LYS 531, ARG 394, TYR 526, LEU 536.The Paracetamol derivatives **5a** having a 1"-benzyl moiety, **5h** having a 1"-benzoyl moiety and **5i** having a 2"-chloro benzyl moiety were also found to be

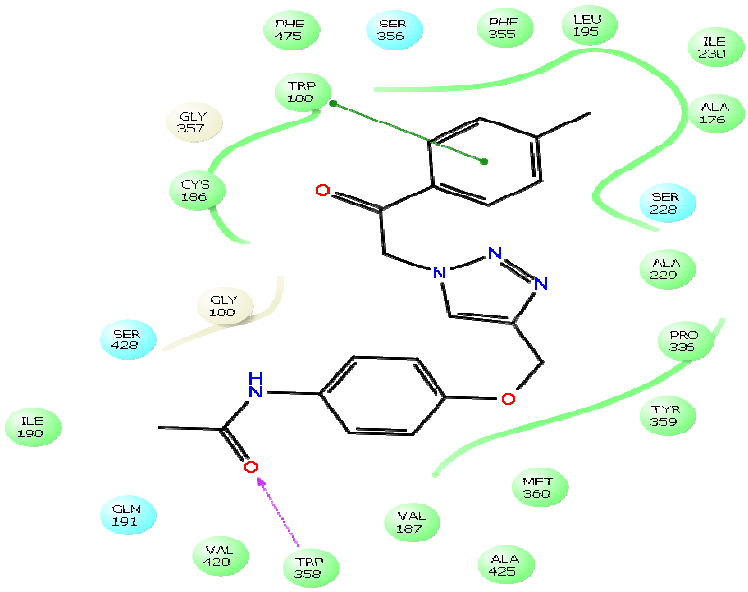


Figure 6 — Interactions (Hydrophobic, Hydrophilic) of **5f** with Amino acids present in the active site of 3NLE.

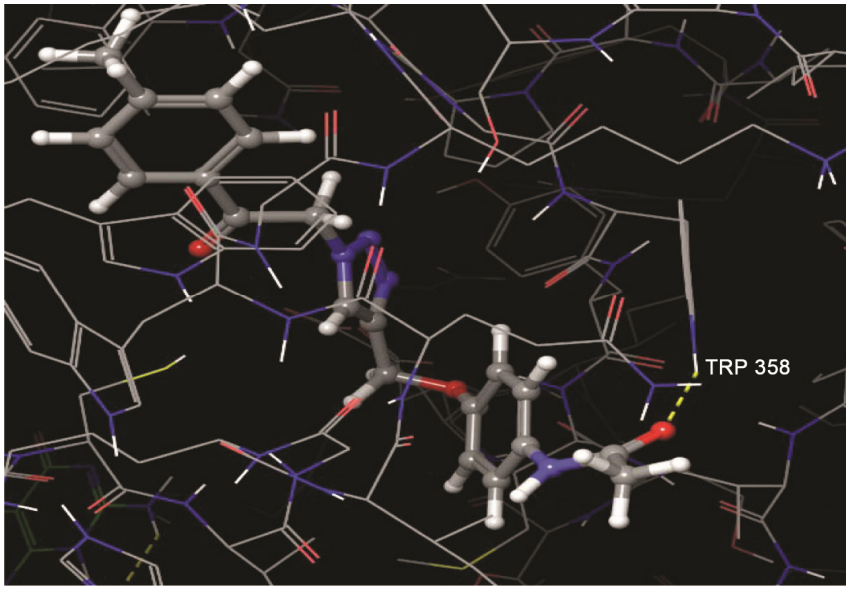


Figure 7 — H-Bonding interactions of **5f** with Amino acids present in the active site of 3NLE.

potent with a IC_{50} value of below 30 µg/mL. All the other Paracetamol derivatives were also found to be potent but IC_{50} value more than 50 µg/mL *i.e.* **5d** having a 4"-chloro benzyl moiety, **5e** having a 3"-chloro benzyl moiety, and **5f** having a 4"-methyl benzoyl moiety.Tamoxifen used as a reference drug.

The Cytotoxic activity of compounds is due to the formation of intermolecular Hydrogen bonds between NH and Amino acids present in the Active site of 1XP6. Phenyl moiety also forms strong Hydrophobic interactions and Cytotoxic activity depends on the R group.

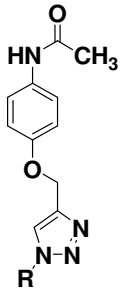
The cytotoxic activity of compound **5a** is due to the formation of intermolecular Hydrogen bonds between NH and LYS 531 and Hydrophobic interaction of Phenyl moiety.

The cytotoxic activity of compounds **5c**, **5g**, **5i** is due to the formation of intermolecular Hydrogen bonds between NH and LYS 531 and TYR 526.Introducing the Fluoro, Nitro, and Chloro groups

Table V — Extra precision (XP) docking results of Schrodinger: Hydrophilic and Hydrophobic Interactions of Compounds with Amino acids of 3NLE.

S.No	Compd	Interacting residues
1	**5a**	TYR 477, ARG 185, TRP 180
2	**5b**	ARG 185, TRP 180, ASN 468, PHE 475, PHE 355
3	**5c**	TYR 477, ARG 185, TRP 180
4	**5d**	PHE 475, PHE 355, TRP 180
5	**5e**	TRP 449, TRP 180
6	**5f**	TRP 358, TRP 180
7	**5g**	PHE 475, TRP 180, PHE 355
8	**5h**	TYR 477, ARG 185, TRP 180
9	**5i**	TYR 477, ARG 185, TRP 180

Table VI — *In vitro* Cytotoxic activity of Paracetamol derivatives (**5a** to **5i**): (Expressed as CTC_{50} in µg/mL).



Compd	R	CTC_{50} in µg/mL
5a	1"-benzyl	30.10
5b	4"-chloro benzoyl	20.83
5c	4"-fluoro benzyl	19.83
5d	4"-chloro benzyl	93.10
5e	3"-chloro benzyl	>100
5f	4"-methyl benzoyl	>100
5g	3"-nitro benzyl	20.57
5h	1"-benzoyl	23.57
5i	2"-chloro benzyl	25.10
Standard Tamoxifen		9.15

on Benzyl moiety also enhances the cytotoxic activity of compounds **5c**, **5g** and **5i**.

The cytotoxic activity of compound **5h** is due to the formation of intermolecular Hydrogen bond between NH and LYS 531. Introducing the Carbonyl group between Methylene and Phenyl also enhances the cytotoxic activity of **5h**.

The cytotoxic activity of compound **5b** is due to the formation of intermolecular Hydrogen bond between NH and LYS 531 and Hydrophobic interaction of Phenyl moiety.Introducing thechloro group on benzoyl moiety also enhances the cytotoxic activity of **5b**.

Table VII — Anti-oxidant activity of Paracetamol derivatives (**5a-5i**) (Expressed as IC_{50} in µg/mL)

Compd	R	IC_{50} in µg/mL
5a	1"-benzyl	1
5b	4"-chloro benzoyl	1
5c	4"-fluoro benzyl	0.9
5d	4"-chloro benzyl	1
5e	3"-chloro benzyl	0.4
5f	4"-methyl benzoyl	0.5
5g	3"-nitro benzyl	0.9
5h	1"-benzoyl	0.9
5i	2"-chloro benzyl	1
Standard (Ascorbic acid)		3

Anti-oxidant activity

From the results it is evident that all the Paracetamol derivatives synthesized, showed good Anti-oxidant activity, comparable with that of the standard. Among the compounds tested, **5e** with 3-Chloro benzyl moiety and **5f** with 4-Methyl benzoyl moiety was found to be the most potent Anti-oxidants and having a IC_{50} value of 0.4 µg/mL and 0.5 µg/mL respectively (Table VII). The Paracetamol derivatives **5c** having a 4-Fluoro benzyl, **5g** having 3-Nitro benzyl substitution and **5h** having Benzoyl substitutionwere also found to be equipotent with a IC_{50} value of 0.9 µg/mL. All the other Paracetamol derivatives in this series were found to be more potent than well-known antioxidant Ascorbic acid in the DPPH-assay.

Conclusion

In summary a new series of N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide derivatives were synthesized by employing Click-Chemistry Approach to target Estrogen receptor Alpha ligand binding domain. The insilico studies showed that N-(4-((1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) acetamide derivatives was found to have good docking score and also showing good Hydrogen bonding interactions with Amino acids present in the active site of Estrogen receptor Alpha ligand binding domain (1XP6). *In vitro* Cytotoxicity of these novel compounds are performed on MCF-7 breast cancer cell line using MTT Assay. Many of the synthesized compounds exhibited potent Cytotoxic activity and **5c**, **5g**, and **5b** compounds showed most potent cytotoxicity with IC_{50} value of 19.83, 20.57, 20.83 µg/mL respectively which is nearer to standard Estrogen receptor positive breast cancer drug Tamoxifen IC_{50} value. **5a**, **5h**, **5i** compounds showed

potent cytotoxicity with IC_{50} value <30 µg/mL. **5d**, **5e**, **5f** compounds showed cytotoxicity with IC_{50} value >90 µg/mL. All Paracetamol derivativeswere found to be more potent than well-known antioxidant Ascorbic acid in the DPPH-assay. **5e** with 3-Chloro benzyl moiety and **5f** with 4-Methyl benzoyl moiety was found to be the most potent Anti-oxidants and having a IC_{50} value of 0.4 µg/mL and 0.5 µg/mL respectively. The Paracetamol derivatives **5c** having a 4-Fluoro benzyl, **5g** having 3-Nitro benzyl substitution and **5h** having Benzoyl substitutionwere also found to be equipotent with a IC_{50} value of 0.9 µg/mL.Thesebiological assay results support that Paracetamol derivatives hold promise as breast cancer agents after further development.

Conflict Of Interest

The authors confirm that this article content has no conflict of interest.

Acknowledgements

The authors are thankful to the management of Vishnu Institute Of Pharmaceutical Education And Research, Narsapur, India for providing necessary facilities. We also thank to Indian Institute Of Chemical Technology, Hyderabad and Radiant Research Services Pvt. Ltd Bangalore for providing ^{1}H NMR, ^{13}C NMR, mass, Infrared spectra and *in vitro* cytotoxicity studies.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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