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Aromatase Inhibitory Activity of Novel Tetrabromoisoindoline Derivatives

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Abstract
Tetrabromophthalic anhydride reacts with p-amino benzoates namely p-amino methyl benzoate and p-amino ethyl benzoate to afford isoindoline derivatives **1a,b**. Also, isoindoline derivative **1a** reacts with hydrazine hydrate to form benzohydrazide derivative **2**. Compound **2** reacts with different aromatic aldehydes namely p-chloro benzaldehyde, p-floro benzaldehyde, and p-N,N-dimethylamino benzaldehyde to afford isoindoline derivatives **3a-c**. Benzohydrazide derivative **2** reacts with ribose, and glucose to form isoindoline derivatives **4a,b**. Compounds **4a,b** is acetylated with acetic anhydride to give acetylated sugar derivatives **5a,b**. Benzohydrazide derivative **2** reacts with potassium thiocyanate to afford triazole derivative **6**. Isoindoline derivative **6** reacts with chloroacetyl chloride to afford thiazole derivative **7** which reacts with p-chlorobenzaldehyde to form isoindoline derivative **8**. Isoindoline derivative **2** reacts with carbon disulfide to afford carbodithioate **9** which interacts with hydrazine hydrate to afford triazole derivative **10**. Trizole derivative **10** reacts with p-chlorobenzaldehyde to afford isoindoline derivative **11**. Benzohydrazide derivative **2** reacts with iosthiocyanate derivatives namely benzylisothiocyanate and phenyl isothiocyanate to give triazole derivative **12a,b**. Aromatase inhibitory effect, and cytotoxic effect against T47D, and MRC-5 of isoindoline derivatives **1a,b-12a,b** were reported.
Keywords
Aromatase inhibitory activity, tetrabromoisoindoline, Tetrabromophthalic anhydride, triazole, dioxoisoindoline, T47D.

1. Introduction

Malignant tumors are considered as main cause for death. It consists of abnormal proliferation of cells that can invade other tissues. 19.3 Million cancer patients were diagnosed in 2020.[1-5] Also, 9.9 million people died from cancer in 2020. Lung cancer is the first cause of death followed by colon, liver, stomach, and breast cancer.[1-5] Cancer patients are expected to increase by 28.4 % in 2040.[1-5] Different cancer therapies are present such as surgery, radiotherapy, and chemotherapy. Chemotherapy has different side effects including low selectivity for cancer cells, drug resistance, and toxicity.[1-5] So that developing more selective and less toxic therapy is the main objective of Medicinal Chemists.

Breast cancer has large amount of aromatase.[6-10] Aromatase inhibitors decrease estrogen secretion which is essential for aromatase. Aromatase inhibitors are classified into four generations according to clinical use order: first, second, third, and fourth generations.[6-12] Also, aromatase inhibitors are classified into two types according to structural constituent; type I (steroidal), type II (nonsteroidal).[6-10] Third generation aromatase inhibitors are selective, least toxic, and most effective e.g. anastrozole, and letrozole (Figure 1).[4-10] Anastrozole, and letrozole contain triazole ring that binds to heme prosthetic group in the aromatase and competitively competes with androgen substrate leading to inhibition of aromatase.[6-10]

1,2,3-Triazole derivatives were discovered to have different biological activities e.g. antiviral activity, antibacterial activity, anti-diabetic activity, anti-Alzheimer activity, anti-inflammatory activity, and anticancer activity.[1] Triazole derivative I inhibits vascular epidermal growth factor receptor 2 with IC_{50} 26.38 nM which is more than sunitinib (IC_{50} 83.2 nM).[1] Triazole derivative II has anticancer activity to HCT116 with IC_{50} 2.6 µM, MCF-7 with IC_{50} 1.1 µM, and HepG2 with IC_{50} 1.4 µM. These activities are higher than reference drug doxorubicin which has IC_{50} 2.5, 1.2, and 1.8 µM against previous cancer cell lines respectively.[1]

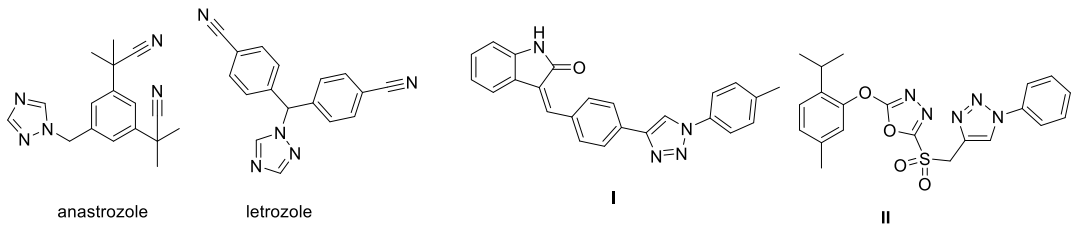

Figure 1: Different biological active triazole derivatives

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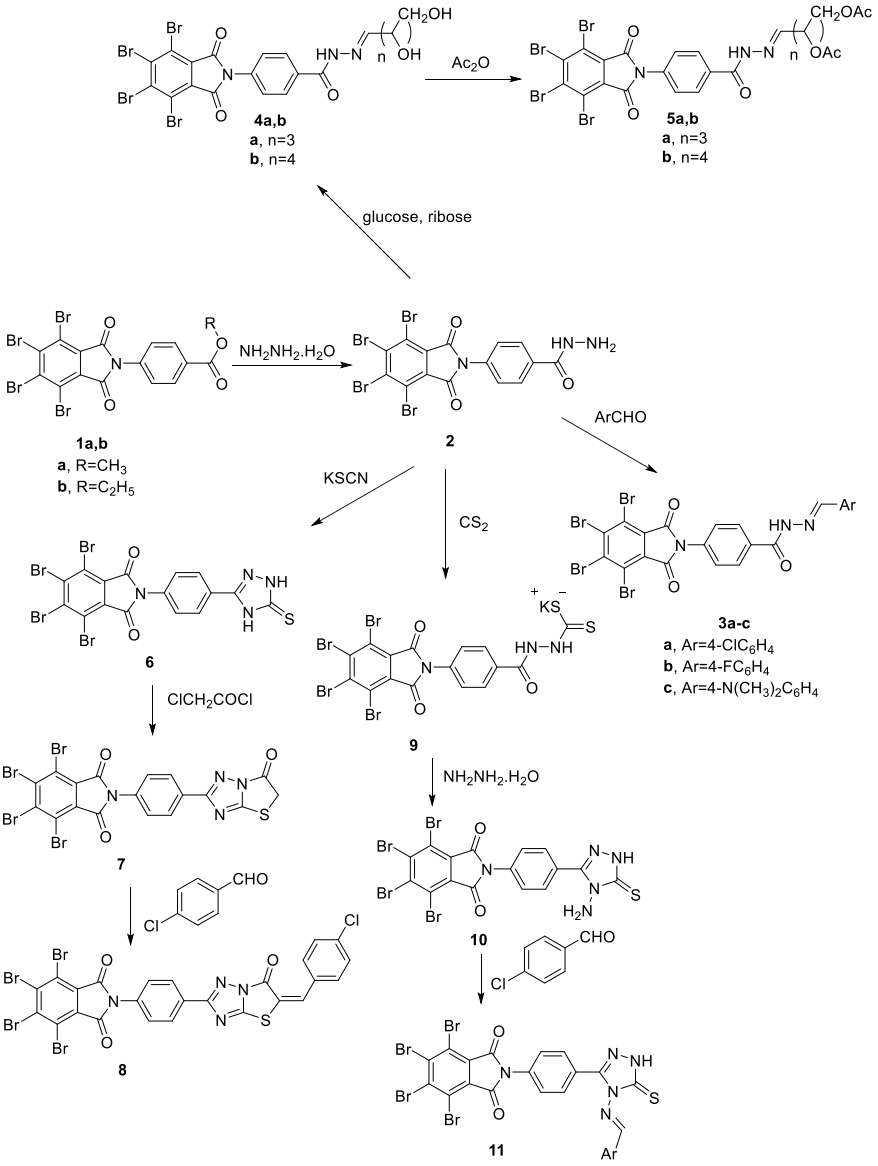
Results and Discussion

Tetrabromophthalic anhydride reacts with p-amino benzoates namely p-amino methyl benzoate and p-amino ethyl benzoate to afford isoindoline derivatives **1a,b**. Also, isoindoline derivative **1a** reacts with hydrazine hydrate to form benzohydrazide derivative **2**. Compound **2** reacts with different aromatic aldehydes namely p-chloro benzaldehyde, p-floro benzaldehyde, and p-N,N-dimethylamino benzaldehyde to afford isoindoline derivatives **3a-c**. Different spectral characterization (MS, IR, ^{1}H & ^{13}C NMR) are in agreement with suggested structures. The IR spectra show appearance of absorption band for two amide function groups in compound **1a** at 1742, and 1655 cm^{-1}. The ^{1}H NMR of compound 1a shows chemical shift characteristic to methyl group at 3.45 ppm. The IR spectrum of compound **2** shows appearance of absorption bands for amino group at 3480 cm^{-1} and appearance of absorption band for additional amide function group. The ^{1}H NMR spectra of compounds **3a-c** show chemical shifts corresponding to phenyl protons and characteristic CH=N protons.

Benzohydrazide derivative **2** reacts with ribose, and glucose to form isoindoline derivatives **4a,b**. Compounds **4a,b** is acetylated with acetic anhydride to give acetylated sugar derivatives **5a,b**. The structures of isoindoline derivatives **4a,b**, and **5a,b** were confirmed through MS, IR, and ^{1}H &^{13}C NMR spectroscopic data. The IR of isoindoline derivative **4a** has absorption band of OH at 3520 cm^{-1}. The ^{1}H NMR of isoindoline derivatives **4a,b** show characteristic chemical shifts corresponding to sugar moiety (CHOH, CH_2OH). The IR spectra of isoindoline derivatives **5a,b** show hydroxyl group absorption band disappearance and ester function group absorption band appearance. The ^{1}H NMR of acetylated sugar derivatives **5a,b** show characteristic chemical shifts corresponding to methyl groups (CH_3CO).

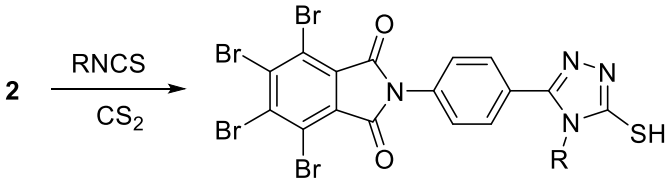


Scheme 1

Benzohydrazide derivative **2** reacts with potassium thiocyanate to afford triazole derivative **6**. Isoindoline derivative **6** reacts with chloroacetyl chloride to afford thiazole derivative **7** which reacts with p-chlorobenzaldehyde to form isoindoline derivative **8**. The spectroscopic data of isoindoline derivatives **6, 7,** and **8** are consistent with the suggested structure. The IR of triazole derivative **6** exhibits disappearance of one amide function group out of three. The MS spectrum of isoindoline derivative **6** show molecular ion peak (M^+). The IR spectrum of isoindoline derivative **7** shows appearance of additional absorption band for amide

function group and amino group absorption band disappearance. Triazole derivative **7** ^{1}H NMR spectrum has characteristic δ corresponding to methyene group (CH_2S). The ^{1}H NMR of triazole derivative **8** shows disappearance of characteristic chemical shift corresponding to methylene group (CH_3S) and appearance of characteristic chemical shift corresponding to CH=.

Isoindoline derivative **2** reacts with carbon disulfide to afford carbodithioate **9** which interacts with hydrazine hydrate to afford triazole derivative **10**. Trizole derivative **10** reacts with p-chlorobenzaldehyde to afford isoindoline derivative **11**. Benzohydrazide derivative **2** reacts with isothiocyanate derivatives namely benzylisothiocyanate and phenyl isothiocyanate to give triazole derivative **12a,b**. The IR spectrum of triazole derivative **10** shows disappearance of absorption band for one amide function groups out of three. The ^{1}H NMR of triazole derivative **11** shows characteristic chemical shift corresponding to CH=. IR spectra of **12a,b** show amino group absorption band disappearance and one amide function group absorption band disappearance out of three. The ^{1}H NMR of isoindoline derivative **12a** shows appearance of characteristic chemical shift corresponding to methylene group (CH_2N).



12a,b

a, R=$CH_2C_6H_5$

b, R=C_6H_5

Scheme 2

Biological activity

The aromatase inhibitory effect of tetrabromoisoindoline derivatives **(1a,b-12a,b)** were measured. Isoindoline derivatives **1a,b,2,3a,5a,b,6,7,8,9,10,11** and **12b** that have inhibition more than 50 % were assessed for their IC_{50} (Table 1). Isoindoline derivatives **3b,c,4a,b,12a** that have inhibition 50 % were considered as inactive compounds (IC_{50} > 12.5M). The low inhibitory activity of isoindoline derivatives **3b,c** can be due to presence of 4-flouro, and 4-N$(CH_3)_2$ substitution in the phenyl group. Also, the low activity of isoindoline derivatives **4a,b** can be due to presence of deacetylated sugar moiety. Presence of benzyl group linked to triazole ring in isoindoline **12a** can be the cause of low aromatase inhibitory activity. The good inhibitory activity of tested compounds towards aromatase can be mainly due to main nucleus which is 4,5,6,7-tetrabromo-isoindoline.

Table 1: Aromatase inhibitory activity of tested compounds, and cytotoxic activity against T47-D, and MRC-5 cell lines

Compound	Cytotoxic activity		
	Aromatase inhibitory	T47-D	MRC-5
1a	0.6 ± 0.25	Non-cytotoxic	Non-cytotoxic
1b	0.58 ± 0.36	19.36 ± 0.879	22.72 ± 3.87
2	2.7 ± 0.25	13.65 ± 3.573	3.55 ± 0.263
3a	0.3 ± 0.5	30.71 ± 3.525	7.36 ± 0.689
3b	>12.5	6.77 ± 1.364	5.33 ± 0.765
3c	>12.5	15.76 ± 0.309	67.48 ± 0.435
4a	>12.5	68.35± 0.387	63.61 ± 1.368
4b	>12.5	Non-cytotoxic	Non-cytotoxic
5a	0.06 ± 0.136	48.67 ± 0.38	Non-cytotoxic
5b	0.89 ± 0.35	Non-cytotoxic	Non-cytotoxic
6	2.8 ± 0.45	Non-cytotoxic	13.28 ± 0.360
7	0.621 ± 0.35	Non-cytotoxic	Non-cytotoxic
8	23.70± 0.21	28.46± 0.24	69.25± 0.38
9	2.35± 0.21	6.25± 0.74	65.23± 0.38
10	0.25 ± 0.25	6.35± 0.26	4.23± 0.23
11	1.25 ± 0.32	Non-cytotoxic	12.38±2.35
12a	>12.5	Non-cytotoxic	Non-cytotoxic
12b	0.5 ± 0.3	33.26 ± 0.639	21.35 ± 0.352
Ketoconazole	2.6 ± 0.7		
Letrozole	0.0019 ± 0.0002		
Etoposide			13.35 ± 0.374
Doxorubicin		0.88 ± 0.021	2.19 ± 0.37

Cytotoxicity assay:
The lethal effect of tested isoindoline derivatives against T47-D cell lines was measured using MTT method (Table 1). The results show that isoindoline derivatives **1b,2,3a,4a,8,9,10,** and **12b** have negligible cytotoxic effect (IC_{50} 6.25-48.67 M). Isoindoline derivatives **3b,c,** and **4a** have cytotoxic effect on T47-D cells but failed to inhibit aromatase (IC_{50} > 12.5 M). Isoindoline derivatives were examined using MTT assay on MTC-5 to measure safety index (Table 1). Isoindoline derivatives **1b,2,3a,8,9,10,** and **12b** have cytotoxic effect on non-cancerous cells at concentrations between 3.55 and 69.25 M.

Experimental

Melting points were measured using Electro-thermal apparatus. Infrared spectra were measured using Perkin-Elmer spectrophotometer. Nuclear magnetic spectroscopy was measured on Jeol-Ex-400 spectrometer. The apparatus used is as reported in a previous paper.[13] The solvent used in the NMR is dimethylsulfoxide.

Preparation of isoindoline derivatives 1a,b
A mixture of tetrabromophthalic anhydride (0.01 mole), and p-amino benzoate (0.01) in 50 mL gl. acetic acid are heated under reflux for 4 hours. The mixture is cooled to r.t.. The precipitate formed is filtered, and crystalized from commercial ethanol to form isoindoline derivative **1a,b**.

Methyl 4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzoate 1a
Yield: 98%; m.p. 260-262 °C; Infrared absorption cm^{-1}, ν: 1742 (C=O), 1655 (C=O); ^{1}H NMR δ/ppm: 3.45 (s, 3H, CH3), 7.56 (d, 2H, j = 7.5 Hz, Ar-H), 8.08 (d, 2H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 45.2 (CH_3), 125.2, 127.2, 130.3, 135.2, 137.5, 138.1, 139.1 (12Ar-C), 159.1 (2 NC=O), 161.5 (C=O). Mass spectroscopy (m/z): 596.8 (M^+, 63%). Calculated elemental analysis for $C_{16}H_7Br_4NO_4$: C, 32.20; H, 1.18; N, 2.35; Found: C, 32.35; H, 1.29; N, 2.49.

Ethyl 4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzoate 1b
Yield: 96%; m.p. 190-192°C; Infrared absorption cm^{-1}, ν: 1745 (CO), 1645 (CO); ^{1}H NMR δ/ppm: 1.30 (t, 3H, j=8 Hz, CH_3), 3.47 (m, 2H, CH_2), 7.56 (d, 2 H, j = 7.5 Hz, Ar-H), 8.07 (d, 2 H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 10.3, 55.2 (CH_3, CH_2), 121.3, 125.2, 127.3, 130.2, 133.5, 135.1, 138.1 (12Ar-C), 157.1 (2 NC=O), 160.5 (C=O). Mass spectrum (m/z): 610.8 (M^+, 55%). Calculated values for elemental analysis for $C_{17}H_9Br_4NO_4$: C, 33.43; H, 1.49; N, 2.29; Found: C, 33.51; H, 1.60; N, 2.35.

4-(4,5,6,7-Tetrabromo-1,3-dioxoisoindolin-2-yl)benzohydrazide 2
A mixture of isoindoline derivative **1a** (0.01 mole), and 1 mL hydrazine hydrate in 50 mL ethanol are refluxed for 4 hours. The reactants are evaporated till dryness. The resulted precipitate crystallized from dioxane to give benzohydrazide derivative **2**.
Yield: 80%; m.p. 300-302 °C; Infrared absortion cm^{-1}, ν: 3450 (NH), 3480 (NH_2), 1682 (CO), 1673 (CO), 1651 (CO) ; ^{1}H NMR δ/ppm: 3.87 (brs, 3H, $NHNH_2$), 7.58 (d, 2 H, j = 7.5 Hz, Ar-H), 8.10 (d, 2 H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 120.3, 122.2, 125.3, 127.2, 130.5, 133.1, 136.1 (12Ar-C), 155.1 (2 NC=O), 158.5 (C=O). Mass spectrum (m/z): 596.8 (M^+, 55%). Elemental analysis calculated values for $C_{15}H_7Br_4N_3O_3$: C, 30.19; H, 1.18; N, 7.04; Found: C, 30.25; H, 1.24; N, 7.10.

General method for preparation of benzohydrazide derivatives 3a-c
A mixture of isoindoline derivative **2** (0.01 mol.), and p-substituted benzaldehyde (0.01 mol.) in 50 mL acetic acid were refluxed for 5 minutes. The solid formed is collected and crystallized from dioxane to give benzohydrazide derivatives **3a-c**.

N'-(4-chlorobenzylidene)-4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzohydrazide 3a
Yield: 98%; m.p. 275-277 °C; Infrared absorption cm^{-1}, ν: 3410 (NH), 1673 (C=O), 1662 (C=O), 1658 (CO) ; ^{1}H NMR δ/ppm: 5.01 (s, 1H, CH=), 7.57 (d, 2H, j = 7.5 Hz, Ar-H), 7.89 (d, 2H, j = 7.5 Hz, Ar-H), 8.08 (d, 2 H, j = 7.5 Hz, Ar-H), 8.20 (d, 2H, j = 7.5 Hz, Ar-H), 9.21 (brs, H, HN). ^{13}C NMR δ/ppm: 121.3, 123.2, 126.3, 128.2, 130.1, 131.1, 134.1, 136.0, 139.3, 140.3, 142.8, 146.1 (19 C=), 158.0 (2 NC=O), 165.5 (C=O). Mass spectrum (m/z): 719.4 (M^+, 51%). Elemental analysis calculated values for $C_{22}H_{10}Br_4ClN_3O_3$: C, 36.73; H, 1.40; N, 5.84; Found: C, 36.79; H, 1.48; N, 5.90.

N'-(4-fluorobenzylidene)-4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzohydrazide 3b
Yield: 96%; m.p. 270-272 °C; Infrared absorption signals cm^{-1}, ν: 3450 (HN), 1681 (C=O), 1675 (C=O), 1655 (CO) ; ^{1}H NMR δ/ppm: 6.45 (s, 1H, CH=), 7.33 (d, 2H, j = 7.5 Hz, Ar-H), 7.57 (d, 2H, j = 7.5 Hz, Ar-H), 7.94 (d, 2 H, j = 7.5 Hz, Ar-H), 8.08 (d, 2H, j = 7.5 Hz, Ar-H), 9.16 (brs, H, HN). ^{13}C NMR δ/ppm: 120.3, 122.0, 125.3, 126.2, 131.3, 133.1, 135.1, 137.2, 139.5, 141.1, 142.9, 144.1 (19 C=), 160.0 (2 NC=O), 167.2 (CO). Mass spectrum signals (m/z): 702.9 (M^+, 51 %). Calculated values for elemental analysis for $C_{22}H_{10}Br_4FN_3O_3$: C, 37.59; H, 1.43; N, 5.98; Found: C, 37.64; H, 1.49; N, 6.04.

N'-(4-(dimethylamino)benzylidene)-4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzohydrazide 3c
Yield: 97%; m.p. 280-282 °C; Infrared absorption cm^{-1}, ν: 3410 (HN), 1672 (C=O), 1663 (C=O), 1646 (CO) ; ^{1}H NMR δ/ppm: 3.29 (s, 6H, $2CH_3$), 5.01 (brs, 1H, NH), 6.72 (s, 1H, CH=), 7.57 (d, 2H, , j = 7.5 Hz, Ar-H), 7.63 (d, 2 H, j = 7.5 Hz, Ar-H),

8.08 (d, 2H, j = 7.5 Hz, Ar-H), 8.10 (d, 2 H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 35 (2 CH_3), 115.3, 120.0, 122.3, 125.1, 130.1, 132.1, 136.1, 138.0, 139.9, 141.5, 143.9, 144.5 (19 C=), 162.0 (2 NC=O), 165.5 (C=O). Mass spectrum (m/z): 728.0 (M^+, 54 %). Calculated elemental analysis for $C_{24}H_{16}Br_4N_4O_3$: C, 39.59; H, 2.22; N, 7.70; Found: C, 39.63; H, 2.29; N, 7.76.

General method for preparation of isoindoline derivatives 4a,b

A mixture of isoindoline derivative **2** (0.01 mole), and different sugars (ribose, and glucose) (0.01 mol.) in 50 mL dioxane having 5 mL acetic acid are refluxed for three hours. The reaction left to reach to room temperature. The formed solid is collected, and crystallized from dioxane to form sugar linked derivatives **4a,b**.

4-(4,5,6,7-Tetrabromo-1,3-dioxoisoindolin-2-yl)-N'-(2,3,4,5-tetrahydroxypentylidene)benzohydrazide 4a

Yield: 66%; m.p. 190-192 °C; Infrared absorption cm^{-1}, ν: 3520 (OH), 3410 (HN), 1681 (C=O), 1675 (C=O), 1661 (CO) ; ^{1}H NMR δ/ppm: 3.28 (t, 1H, j=7 Hz, CHOH), 3.30 (brs, 4 H, 4OH), 3.46 (t, 1H, j=7 Hz, CHOH), 3.86 (m, 1H, CHOH), 4.31 (d, 2 H, j = 7 Hz, CH_2OH), 6.72 (d, 1H, j = 6.2 Hz, CH=), 7.56 (d, 2 H, j = 7.5 Hz, Ar-H), 7.99 (d, 2 H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 34.1, 37.5, 40.2, 43.5 (3 CH, CH2), 118.3, 121.0, 122.5, 125.4, 128.4, 130.2, 135.4, 137.0, 140.9, 141.8, 143.1, 145.5 (19 C=), 163.0 (2 NC=O), 167.5 (C=O). Mass spectrum (m/z): 728.9 (M^+, 61%). Calculated elemental analysis for $C_{20}H_{15}Br_4N_3O_7$: C, 32.95; H, 2.07; N, 5.76; Found: C, 33.01; H, 2.13; N, 5.81.

N'-(2,3,4,5,6-pentahydroxyhexylidene)-4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzohydrazide 4b

Yield: 68%; m.p. 200-202 °C; Infrared absorption cm^{-1}, ν: 3510 (OH), 3480 (HN), 1678 (C=O), 1664 (C=O), 1658 (CO) ; ^{1}H NMR δ/ppm: 3.02 (t, 1H, j=7 Hz, CHOH), 3.20-3.60 (brs, 5H, 5 OH), 3.29 (t, H, j = 7 Hz, CHOH), 3.40 (t, H, j = 7 Hz, CHOH), 3.50 (m, 1 H, CHOH), 3.56 (d, 1H, j=7 Hz, CH_2OH), 6.67 (d, H, j = 6.2 Hz, CH=), 7.56 (d, 2 H, j = 7.5 Hz, Ar-H), 8.06 (d, 2 H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 31.5, 35.3, 36.5, 41.2, 44.5 (4 CH, CH_2), 115.1, 118.0, 120.5, 122.6, 125.1, 127.2, 130.4, 134.1, 137.9, 140.8, 142.1, 146.7 (19 C=), 160.2 (2 NC=O), 163.5 (C=O). Mass spectrum (m/z): 759.0 (M^+, 61%). Calculated elemental analysis for $C_{21}H_{17}Br_4N_3O_8$: C, 33.23; H, 2.26; N, 5.54; Found: C, 33.29; H, 2.31; N, 5.61.

General method for synthesis of acetylated sugar derivatives 5a,b

A mixture of isoindline derivatives **4a,b** (0.01 mole), and 15 mL acetic anhydride in 30 mL ethanol are heated under reflux for six hours. The reactants are concentrated to its half. The resulted solid is filtered, dried, and recrystallized from commercial ethanol to form isoindoline derivatives **5a,b**.

5-(2-(4-(4,5,6,7-Tetrabromo-1,3-dioxoisoindolin-2-yl)benzoyl)hydrazono)pentane-1,2,3,4-tetrayl tetraacetate 5a

Yield: 58%; m.p. 205-207 °C; Infrared spectrum cm^{-1}, ν: 3420 (HN), 1710 (CO), 1682 (CO); ^{1}H NMR (DMSO) δ/ppm: 0.07 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 2.03 (s, 3 H, CH_3), 2.40 (t, 1H, j = 7 Hz, CHOAc), 3.36 (t, 1H, j = 7 Hz, CHOAc), 3.45 (brs, 1H, NH), 3.52 (m, 1H, CHOAc), 4.30 (d, 2H, j=7 Hz, CH_2OAc), 6.72 (d, H, j = 6.2 Hz, CH=), 7.57 (d, 2 H, j = 7.5 Hz, Ar-H), 8.08 (d, 2H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 15.4, 18.7, 20.1, 24.8 (4CH_3), 30.1, 34.5, 37.2, 40.5 (3 CH, CH_2), 116.1, 120.0, 123.5, 126.4, 129.4, 131.2, 136.4, 138.0, 141.9, 145.8, 147.5, 147.8 (19 C=), 158.4 (2 NC=O), 162.5 (C=O). Mass spectrum (m/z): 897.1 (M^+, 55%). Calculated values for elemental analysis for $C_{28}H_{23}Br_4N_3O_{11}$: C, 37.49; H, 2.58; N, 4.68; Found: C, 37.55; H, 2.64; N, 4.73.

6-(2-(4-(4,5,6,7-Tetrabromo-1,3-dioxoisoindolin-2-yl)benzoyl)hydrazono)hexane-1,2,3,4,5-pentayl pentaacetate 5b

Yield: 55%; m.p. 250-252 °C; Infrared absorption cm^{-1}, ν: 3430 (HN), 1742 (CO), 1653 (CO); ^{1}H NMR (DMSO) δ/ppm: 1.31 (s, 1H, CH_3), 1.91 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.05 (s, 3 H, CH_3), 2.46 (s, 3H, CH_3), 3.29 (t, H, j = 7 Hz, CHOAc), 3.40 (t, H, j = 7 Hz, CHOAc), 3.53 (t, H, j = 7 Hz, CHOAc), 3.55 (m, H, CHOAc), 3.86 (d, 2H, j = 7 Hz, CH_2Ac), 6.70 (d, H, j = 6.2 Hz, CH=), 7.56 (d, 2H, j = 7.5 Hz, Ar-H), 8.08 (d, 2 H, j = 7.5 Hz, Ar-H), 10.77 (brs, 1 H, HN). ^{13}C NMR (DMSO) δ/ppm: 16.1, 18.4, 19.2, 20.5, 22.0 (5CH_3), 30.5, 32.3, 34.5, 40.2, 42.5 (4 CH, CH_2), 112.1, 115.0, 119.5, 120.6, 122.1, 125.2, 127.4, 130.1, 135.9, 138.8, 140.1, 142.7 (19 C=), 156.2 (2 NCO), 161.5 (CO). Mass spectrum (m/z): 969.1 (M^+, 58%). Calculated elemental analysis $C_{31}H_{27}Br_4N_3O_{13}$: C, 38.42; H, 2.81; N, 4.34; Found: C, 38.49; H, 2.87; N, 4.41.

4,5,6,7-Tetrabromo-2-(4-(5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)phenyl)isoindoline-1,3-dione 6

A mixture of isoindoline **2** (0.01 mole), and potassium thiocyanate (1.5 gm) in 5 mL conc. HCl is dissolved in 50 mL dioxane. The reactants are refluxed for one hour. The reactants are evaporated under vacuum. The acidity is neutralized with potassium hydroxide (1.5 gm) in 50 mL dioxane. The new mixture is refluxed for six hours. Then, the reactants are acidified with cold HCl. The formed solid is filtered, dried, and recrystallized from commercial ethanol to form triazole derivative **6**.

Yield: 73%; m.p. 290-292 °C; Infrared spectrum cm^{-1}, ν: 3450 (HN), 1670 (CO), 1666 (CO); ^{1}H NMR δ/ppm: 7.18 (d, 2H, j = 7.5 Hz, Ar-H), 7.30 (d, 2H, j = 7.5 Hz, Ar-H), 14.25 (brs, 2H, 2NH). MS (m/z): 637.9 (M^+, 59%). ^{13}C NMR (DMSO) δ/ppm: 117.5, 119.2, 121.5, 123.6, 126.1, 128.2, 132.4, 141.1 (13 C=), 158.2 (2 NC=O), 171.5 (C=S). Calculated values for elemental analysis for $C_{16}H_6Br_4N_4O_2S$: C, 30.13; H, 0.95; N, 8.78; Found: C, 30.19; H, 1.03 N, 8.85.

4,5,6,7-Tetrabromo-2-(4-(6-oxo-5,6-dihydrothiazolo[3,2-b][1,2,4]triazol-2-yl)phenyl)isoindoline-1,3-dione 7

Mixture of isoindoline derivative **6** (0.01 mole), and chloroacetic acid (0.01 mol.) is dissolved in acetic acid (30 mL), acetic anhyd. (30 mL), and anhydrous sodium acetate (10 gm). The reaction mixture refluxed for 3 hours. The reactants are left to reach room temperature, and added into cold water. Solid formed is filtered, dried, and recrystallized from commercial ethanol to afford isoindoline derivative **7**.

Yield: 75%; m.p. 230-232 °C; Infrared absorption cm^{-1}, ν: 1682 (CO), 1671 (CO), 1655 (CO); ^{1}H NMR (DMSO) δ/ppm: 3.37 (s, 2H, CH_2), 7.15 (d, 2H, j = 7.5 Hz, Ar-H), 7.21 (d, 2H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 21.3 (CH_2), 116.2, 121.1, 123.3, 126.9, 128.1, 130.2, 133.9, 145.1, 148.7 (14C=), 160.5 (2C=O), 171.3 (C=O). Mass spectrum (m/z): 677.9 (M^+, 61%). Calculated values for elemental analysis for $C_{18}H_6Br_4N_4O_3S$: C, 31.89; H, 0.89; N, 8.26; Found: C, 31.95; H, 0.94; N, 8.33.

4,5,6,7-Tetrabromo-2-(4-(5-(4-chlorobenzylidene)-6-oxo-5,6-dihydrothiazolo[3,2-b][1,2,4]triazol-2-yl)phenyl)isoindoline-1,3-dione 8

Mixture of thiazole derivative **7** (0.01 mole), and p-chlorobenzaldehyde (0.01 mol.) is dissolved in acetic acid (30 mL), acetic anhyd. (30 mL), and anhydrous sodium acetate (10 gm). The reaction mixture is refluxed for three hours. Then, the reactants are left to reach room temperature and poured into cold water. The resultant solid is filtered, dried, and recrystallized from commercial ethanol to afford triazole derivative **8**.

Yield: 73%; m.p. 300-302 °C; Infrared spectrum cm^{-1}, ν: 1687 (NH), 1675 (CO), 1658 (CO); ^{1}H NMR (DMSO) δ/ppm: 6.51 (s, 1H, CH=), 7.42 (d, 2H, j = 7.5 Hz, Ar-H), 7.50 (d, 2H, j = 7.5Hz, Ar-H), 7.65 (d, 2 H, j = 7.5 Hz, Ar-H), 7.91 (d, 2H, j = 7.5 Hz, Ar-H). ^{13}C NMR (DMSO) δ/ppm: 115.2, 118.1, 120.3, 122.9, 123.1, 126.4, 128.9, 131.5, 134.7, 136.1, 137.3, 138.2, 138.7, 139.0, 139.5, 139.8, 140.1, 140.5, 141.1, 141.6, 147.2, 152.3 (22C=), 165.3 (2C=O), 170.4 (C=O). Mass spectrum (m/z): 800.5 (M^+, 67%). Calculated values for elemental analysis for $C_{25}H_9Br_4ClN_4O_3S$: C, 37.51; H, 1.13; N, 7.00; Found: C, 37.58; H, 1.19; N, 7.10.

Potassium 2-(4-(4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)benzoyl)hydrazine-1-carbodithioate 9

A mixture of isoindoline derivative **2** (0.01 mole), and carbon disulfide (2 mL) in 50 mL dioxane is refluxed for 0.5 hour. The reactants are evaporated under vacuum. The solid formed is filtered, dried, and recrystallized from commercial ethanol to afford carbodithioate derivative **9**.

Yield: 78%; Infrared absorption cm^{-1}, ν: 3410 (NH), 1673 (CO), 1665 (CO), 1648 (CO); ^{1}H NMR (DMSO) δ/ppm: 3.40 (brs, 2H, 2NH), 7.09 (d, 2H, j = 7.5 Hz, Ar-H), 7.20 (d, 2H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 116.4, 119.1, 122.3, 125.9, 127.4, 130.2, 133.9 (12C=), 156.3 (2C=O), 162.1 (C=O), 175.2 (C=S). Mass spectrum (m/z): 711.0 (M^+, 71%). Calculated elemental analysis for $C_{16}H_6Br_4KN_3O_3S_2$: C, 27.03; H, 0.85; N, 5.91; Found: C, 27.10; H, 0.97; N, 6.05.

2-(4-(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)phenyl)-4,5,6,7-tetrabromoisoindoline-1,3-dione 10

A mixture of isoindoline derivative **9** (0.01 mole), and hydrazine hydrate (1 mL) are dissolved in water (50 mL). The reaction mixture refluxed for six hours. The reactants are left to reach room temperature. Then, cold HCl is poured to the reactants. The formed precipitate is collected, dried, and recrystallized from commercial ethanol to form triazole derivative **10**.

Yield: 70 %; m.p. > 300 °C; Infrared absorption cm^{-1}, ν: 3410 (HN), 1667 (CO), 1653 (CO); ^{1}H NMR (DMSO) δ/ppm: 6.71 (brs, 3H,$NHNH_2$), 7.24 (d, 2H, j = 7.5 Hz, Ar-H), 7.34 (d, 2H, j = 7.5 Hz, Ar-H). ^{13}C NMR δ/ppm: 119.1, 122.1, 124.3, 126.9, 128.1, 129.2, 130.9, 141.7 (13C=), 158.3 (C=O), 172.5 (C=S). Mass spectrum (m/z): 652.9 (M^+, 54%). Calculated elemental analysis for $C_{16}H_7Br_4N_5O_2S$: C, 29.43; H, 1.08; N, 10.73; Found: C, 29.51; H, 1.13; N, 10.80.

4,5,6,7-Tetrabromo-2-(4-(4-((4-chlorobenzylidene)amino)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)phenyl)isoindoline-1,3-dione 11

A mixture of triazole derivative **10** (0.01 mol.), and p-chlorobenzaldehyde (0.01 mol.) are dissolved in acetic acid (20 mL). The reactants are refluxed for five minutes. The formed solid is collected, and recrystallized from commercial ethanol to form isoindoline derivative **11**.

Yield: 50 %; m.p. > 300 °C; Infrared absorption cm^{-1}, ν: 3410 (HN), 1671 (CO), 1655 (CO); ^{1}H NMR (DMSO) δ/ppm: 2.46 (brs, 1H, NH), 7.52 (d, 2H, j = 7.5 Hz, Ar-H), 7.84 (d, 2H, j = 7.5 Hz, Ar-H), 8.66 (d, H, CH=). ^{13}C NMR δ/ppm: 119.4, 122.1, 124.5, 126.9, 128.1, 130.2, 133.9, 135.1, 136.1, 137.1, 139.3, 143.2, 148.7 (20C=), 160.3 (2C=O), 173.5 (C=S). Mass spectrum (m/z): 775.4 (M^+, 67%). Calculated elemental analysis for $C_{23}H_{10}Br_4ClN_5O_2S$: C, 35.62; H, 1.30; N, 9.03; Found: C, 35.69; H, 1.38; N,9.10.

General method for preparation of triazole derivatives 12a,b

Mixture of isoindoline derivative **2** (0.01 mol.), and isothiocyanate derivatives (0.01 mol.) are dissolved in dioxane (50 mL). The reaction mixture is heated under reflux for six hours. Potassium hydroxide (1gm) in water (10 mL), and carbon disulfide (2 mL) are poured to reactants. The reactants are refluxed again for six hours. Then, the reaction mixture is concentrated to its half volume. Cold HCl is poured to the reactants. The formed solid is collected, and crystallized from commercial ethanol to form triazole derivative **12a,b**.

2-(4-(4-Benzyl-5-mercapto-4H-1,2,4-triazol-3-yl)phenyl)-4,5,6,7-tetrabromoisoindoline-1,3-dione 12a

Yield: 60 %; m.p. 270-272 °C; Infrared absorption cm^{-1}, ν: 1675 (C=O), 1651 (C=O); ^{1}H NMR δ/ppm: 3.52 (s, 2H, CH_2), 4.43 (brs, H, SH), 7.17-7.38 (m, 9H, Ar-H). ^{13}C NMR δ/ppm: 30.6 (CH_2), 118.2, 120.5, 121.4, 122.6, 123.1, 125.2, 127.9, 129.1, 130.7, 132.1, 135.3, 145.2, 154.7 (20C=), 163.3 (2C=O). Mass spectrum (m/z): 728.0 (M^+, 61%). Calculated elemental analysis for $C_{23}H_{12}Br_4N_4O_2S$: C, 37.94; H, 1.66; N, 7.70; Found: C, 38.01; H, 2.70; N, 7.78.

4,5,6,7-Tetrabromo-2-(4-(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)phenyl)isoindoline-1,3-dione 12b

Yield: 65 %; m.p. 240-242 °C; Infrared spectrum cm^{-1}, ν: 1676 (C=O), 1651 (CO); ^{1}H NMR δ/ppm: 6.90 (brs, 1H, SH), 7.26 (d, 2H, j = 7.5 Hz, Ar-H), 7.40 (d, 2H, j = 7.5 Hz, Ar-H), 7.51 (m, 5H, Ar-H). ^{13}C NMR δ/ppm: 119.2, 121.5, 122.4, 124.6, 125.1, 126.2, 127.9, 129.5, 130.8, 133.1, 136.3, 149.2, 158.7 (20C=), 161.3 (2C=O). Mass spectrum (m/z): 714.0 (M^+, 61%). Calculated elemental analysis for $C_{22}H_{10}Br_4N_4O_2S$: C, 37.01; H, 1.41; N, 7.85; Found: C, 37.10; H, 1.48; N, 7.94.

Biological activity

Aromatase inhibition assay

Stresser et al. method was used to measure aromatase inhibitory effect with minor modifications.[14,15] The previous method uses fluorometric substrates CYP19 enzyme and DBF. Cofactor (which have buffer of phosphate, system that generates NADPH, and glucose-6-phosphate dehydrogenase) was pipetted to well plate. The reaction started by adding 100 µL of substrate / enzyme mixture that contains 12.5 µL of 16 pmol/mL CYP19, buffer of phosphate, tested sample or 10% dimethylsulfoxide or letrozole/ ketoconazole, and 0.2 µL of 0.2 mM DBF. The fluorescence signal is measured using 490 nm excitation wavelength, 515 nm cutoff wavelength, and 530 nm emission wavelength. Percent inhibition was measured using equation 1. Tested samples that have more than fifty % inhibition were diluted and measured. A graph containing concentration versus % inhibition is used to determine IC_{50}.

$$\% \text{ inhibition} = 100\ [(\text{blank sample})/(\text{blank DMSO}) \times 100] \quad \text{(Equation 1)}$$

Cytotoxicity assay

Cytotoxic profile of tested compounds **(1a,b-12a)** was measured using hormone dependant breast cancer cell line (T47-D), and typical embryonic lung cell line (MRC-5). T47-D cell line were grown with 100 U/mL penicillin-streptomycin, 2mM L-gluthamine, 0.2 U/mL insulin, 10 % FBS, and 4.5 g/L glucose. MRC-5 cell lines were grown supported with FBS, and penicillin-streptomycin. Cell lines present in suitable culture media were inoculated into well microtiter plates at density of 100000-20000 cells per well. Then, the plates were incubated with CO_2, and air for 24 hours. Equivalent amount of additional medium that consists of either successive dilutions of the samples being examined, dimethylsulphoxide as negative control, or etoposide or doxorubicin as positive control was added to required concentrations. Additional fourty eight hours period of incubation was done. Cells viability was measured through MTT assay.[16] Solution of MTT (10 mL) was poured to each assay's well, then, plates incubated for 2-4 hours. Resulting formazan was sonicated to be dissolved before dimethylsulfoxide was added. Plates were measured using microplate reader (produced by Molecular devices, U.S.A.) at 550 nm and reference wavelength

650 nm. IC_{50} is a concentration of a substance in which growth of cells was 50 % inhibited. If IC_{50} is higher than 50 g/mL, the substance is considered as noncytotoxic.

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Conflict of interest

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