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Synthesis of New Chlorambucil Derivatives with Expected Antitumor Activity.

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ABSTRACT

2-Acetyl-3-(4-(4-[bis(2-chloroethyl) amino] phenylbutanoyl) tetrazolidine-5-thione (**2**) was prepared in moderate yield by reaction of compound **1** in acetic anhydride. 4-(4-[bis(2-chloroethyl) amino]phenyl-N-[(2-cyanoacetyl carbamoyl] butanamide (**3**) was obtained at refluxing of compound (**x**) and cyanoacetyl urea in absolute alcohol. 4-(bis(2-chloroethyl)amino derivatives (**4,5**) was prepared by reaction of thioglycolic acid and/or salisaldehyde in presence of glacial acetic acid with compound **3**. while the reaction of compound **3** with *o*-amino phenol, *o*-phenylene diamine and/ or *o*-amino thiophenol in ethanol, was yielded a compound derivatives **6-8**. Also, 4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(2-cyano-3 (dimethylamino) acryloyl carbamoyl)butanamide (**9**) was obtained at refluxing with compound **3** and *N,N*-dimethyl formamide dimethyl acetal in dry toluene. At the reaction of cyclohexanone and ammonium acetate in chloroform with compound **3**, 4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(2-cyano-2-cyclohexylideneacetylcarbamoyl)butanamide (**10**) was obtained. While at the reaction of *p*-fluorobenzaldehyde in absolute ethanol with compound (**3**), 4-(4-(bis(2-chloroethyl)amino)phenyl)-N-2-cyano-3-(4-fluorophenylacryloylcarbamoyl) butanamide (**11**) was obtained. The anticancer activity results indicated that the synthesized products **2, 4, 5, 7, 8** and **x** showed growth inhibition activity against the tested one cell line but with varying intensities extents in comparison to the known anticancer drugs Doxorubicin.

Keywords: Tetrazolidine-5-thione, Butanamide, *p*-Fluorobenzaldehyde, Antitumour activity.

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INTRODUCTION

Cancer is a major health problem worldwide. Improvements in treatment and prevention have led to a decrease in cancer deaths, but the number of new diagnoses continues to rise. Chemotherapy is one of the most commonly used treatment options. So it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. Thus, it is urgent to develop novel chemotherapeutic agents for the treatment of cancer. Chlorambucil[1-10] or 4-(4-(Bis(2-chloroethyl)amino)phenyl)butanoic acid is a bifunctional aromatic derivative of nitrogen mustard (Fig. 1). Alkylating agents, such as chlorambucil, are extensively used in the treatment of neoplastic diseases, but their effectiveness is often limited by the emergence of drug resistant tumor cells. Its mode of action has not, as yet, been entirely clarified. Previously, researchers attributed chlorambucil's cytotoxicity solely to its inhibitory effects on DNA synthesis.[11] More recent studies have revealed that chlorambucil indirectly affects the synthesis of DNA.[12] Biochemical studies have found that chlorambucil affects other cellular constituents as well,[13] especially nuclear proteins.[14] Singlet electron transfer methodology is emerging as an exciting new technology for mild and practical synthesis of a large number and highly branched compounds via SRN₁, bis-SRN₁, ERC₁, LD-SRN₁... mechanisms.[15] Reductive-halogenation reactions of halogenated ketones using Rongalite,[16] sodium dithionite (Na₂S₂O₄),[17] led to the formation of an anion via a SET.

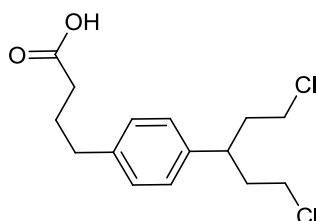


Figure 1: Structure of Chlorambucil

In continuation of our program directed toward the study of singlet electron transfer (SET) reactions[15,18] of bio reductive alkylating agents and the development of new analogs of chlorambucil as anticancer agents. The diaryl ethanol compounds could be suitable intermediates of the synthesis of chlorambucil analogs after reduction of nitro and alkylation. Generally, these derivatives were prepared via the reaction of benzyl magnesium bromide or chloride with various aromatic aldehydes.[19] Moreover, Tanaka[20] reported a new combination of TDAE, as organic reducing agent, and transition metal catalysts (Cr, Ni) for allylation of aldehydes and ketones. A great deal of work devotes to the study of chlorambucil derivatives since this moiety is found in a wide variety of synthetic. As a continuation to our previous work in synthesizing antitumor chlorambucil compounds, new chlorambucil derivatives **2**, **4**, **5**, **7**, **8** and **x** were prepared. The antitumor effect of the compounds **2**, **4**, **5**, **7**, **8** and **x** was studied against breast cancer (MCF7) and compound **4** [IC₅₀=5.74 MM] was found to be more active than doxorubicin.

METHODOLOGY

Cytotoxicity Assessment:

Cell culture

MCF-7 human breast cancer cells was grown in RPMI-1640 medium, supplemented with 10% heat inactivated FBS, 50 units/mL of penicillin and 50 g/mL of streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO₂. The cells were maintained as "monolayer culture" by serial sub-culturing.

SRB cytotoxicity assay

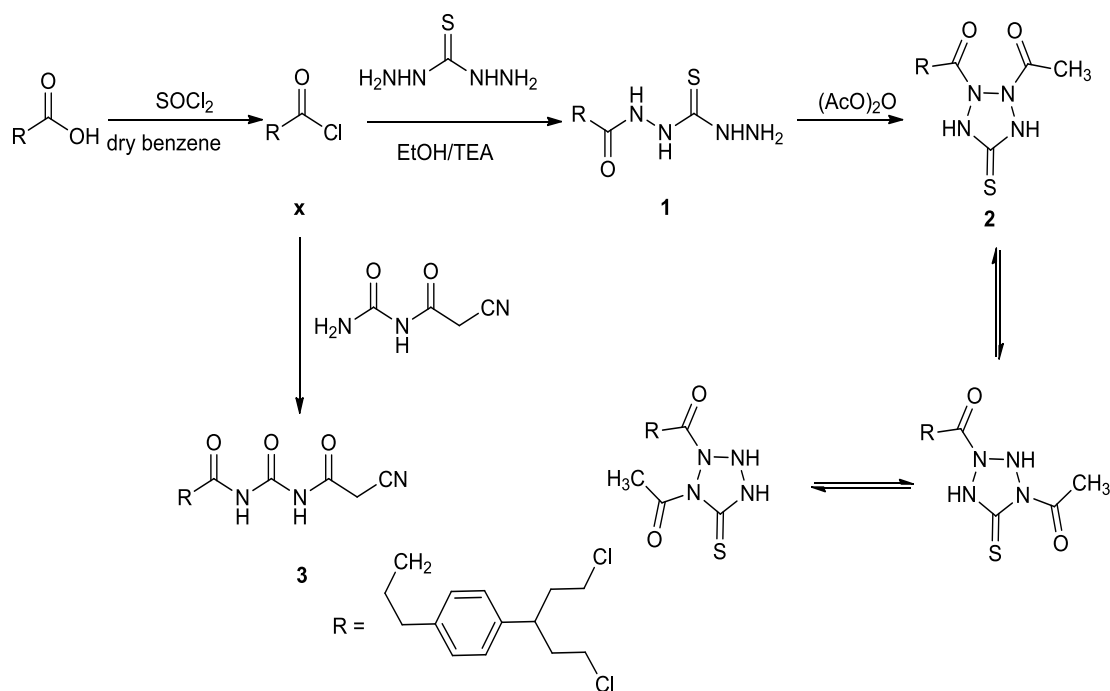
Cytotoxicity was determined using SRB method as previously described[21-23]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000-2000 cells/well in RPMI-1640 supplemented medium. After 24 h, cells were incubated for 72 h with various concentrations of the tested compounds. Following 72 h treatment, the cells will be fixed with 10% trichloroacetic acid for 1 h at 4 °C. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates

were air dried for 24 h and the dye was solubilized with Tris-HCl for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometric-ally at 564 nm with an ELISA micro-plate reader (ChroMate-4300, FL, USA). The IC_{50} values were calculated according to the equation for Boltzmann sigmoidal concentration–response curve using the nonlinear regression fitting models

RESULTS AND DISCUSSION

Chemistry

4-(4-(Bis(2-chloroethyl)amino)phenyl) butanoic acid (chlorambucil) was reacted with thionyl chloride in presence of dry benzene, 4-(4-(bis(2-chloroethyl)amino)phenyl) butanoyl chloride (**x**) was obtained (Scheme 1). The IR spectra of compound (**x**), it was observed the absent of OH group of (COOH) and the MS of compound (**x**) showed m/z 322.5 (M^+). 4-(4-(bis(2-chloroethyl) amino) phenyl)-N-(hydrazine carbonothionyl) butane hydrazide (**1**) (Scheme 1) was obtained at the reaction compound (**x**) with thiocarbohydrazide in the presence of absolute ethanol and drops of triethylamine. The IR spectra of compound (**1**) showed the absorption band at 3400-3200 (3NH, NH_2), 1699 (C=O) and 1219 (C=S). Compound (**1**) reacted with acetic anhydride to obtain 2-acetyl-3-(4-(4-[bis(2-chloroethyl) amino] phenyl) butanoyl) tetrazolidine-5-thione (**2**) (Scheme 1). The 1H NMR spectrum of compound (**2**) revealed the presence of singlet signal at δ = 2.00 (CH_3), broad signal at 8.40, 9.20 (2NH, D_2O exchangeable). Also compound (**x**) was reacted with cyanoacetyl urea in the presence of absolute ethanol and triethyl amine to obtain 4-(4-[bis(2-chloroethyl)amino]phenyl)-N-[(2-cyanoacetylcarbamoyl)]butanamide (**3**) (Scheme 1). The 1H NMR spectrum of compound (**3**) was revealed the presence of singlet signal at δ = 4.30 (CH_2CN), broad signal at 9.70 (2NH $_2$, D_2O exchangeable).



Scheme 1

(4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(2-(4-hydroxythiazol-2-yl)acetyl) carbamoyl) butanamide (**4**) (Scheme 2) was obtained at the refluxing of compound **3** with thioglycolic acid in presence of glacial acetic acid²⁴. The IR spectra of compound **4** showed the absorption band at 3400 (OH). The 1H NMR spectrum of compound **4** showed singlet signal at δ = 7.23 (=CH, thiazole), singlet signal at δ = 12.10 (OH, D_2O exchangeable). Also compound **3** was reacted with salisaldehyde, in presence of glacial acetic acid to yield N-(4-(4-(bis(2-chloroethyl) amino) phenyl) butanoylcarbamoyl)-2-imino-2H-chromene-3-carboxamide (**5**) (Scheme 2). The 1H NMR spectrum of compound **5** showed singlet signal at δ = 8.53 (CH of chromene). MS of compound (**5**) showed m/z 518 ($M^+ + 1$). When compound **3** was reacted with *o*-amino phenol, *o*-phenylenediamine and /or *o*-amino thiophenol in presence of ethanol and few drops of piperidine-N-(4-(4-(bis(2-chloroethyl)amino)

phenyl)butanoyl) derivatives **6-8** were obtained (Scheme 2). The MS of compound **6** displayed m/z 507 ($M^+ + 2$), IR spectrum showed the absorption band at 3125-3110 (2NH), 1694, 1683, 1670 (3 C=O) and 1654 (C=N). $^1\text{H NMR}$ of compound **7** revealed the presence of multiplet signal at 7.20-7.26 (2H of benzimidazol), multiplet signal at 7.81-7.85 (2H of benzimidazole), broad signal at 9.90 (2NH₂, D₂O exchangeable), and singlet signal at 4.35 (CH₂-benzimidazol). The MS of compound **8** showed m/z 523 ($M^+ + 2$). The IR spectra of compound **8** showed the absorption band at 3065-3000 (2NH), 1710, 1695, 1680 (3C=O) and 1648 (C=N).

4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(2-cyano-3(dimethylamino)acryloyl carbamoyl) butanamide (**9**) was obtained (Scheme 1), When compound **3** was reacted with DMF-DMA in presence of dry toluene - $^1\text{H NMR}$ of compound **9** was revealed the presence of singlet t signal at 7.40 (=CH), 2 singlet t signal at 3.14, 3.22 (2CH₃). Also compound **3** was reacted with cyclohexanone in presence of ammonium acetate, dry chloroform and few drops of glacial acetic acid. 4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(2-cyano-2-cyclohexylideneacetylcarbamoyl)butanamide (**10**) was obtained. The MS of compound **10** displayed m/z 494 ($M^+ + 1$), also compound (**3**) was reacted with *p*-flourobzaldehyde in presence of absolute ethanol and few drops of glacial acetic acid to obtain 4-(4-(bis(2-chloroethyl)amino)phenyl)-N-2-cyano-3-(4-Fluorophenylacryloylcarbamoyl)butanamide (**11**). Compound **11** was revealed the presence of singlet t signal at $\delta = 7.60$ (=CH), multiplet signal at 6.86-7.10 (8H of 2Ar), MS of this compound showed m/z 520 ($M^+ + 1$).

The assignments of all newly synthesized compounds were confirmed by their different spectral data such as $^1\text{H NMR}$, IR, mass spectrum and micro analysis. See experimental.

Cytotoxicity

Compounds **x**, **2**, **4**, **5**, **7** and **8** were studied for their antitumor activity against breast cancer cell line MCF7. Compounds **7**, **8** and **5** have moderate activity in a decreasing order and the activity of compound **x** was weak. While compound **4** was found to be highly potent than doxorubicin and compound **8** was active it was believed the thiazole ring was increased the activity (Table 1).

Table 1: IC₅₀ (μM) values of compounds **2**, **4**, **5**, **7**, **8** and **x** against breast cancer cell

Compound no.	IC ₅₀ (μM)
8	29.90
4	5.74
5	41.54
2	33.40
7	35.19
Start x	47.45
Doxorubicin	17.12

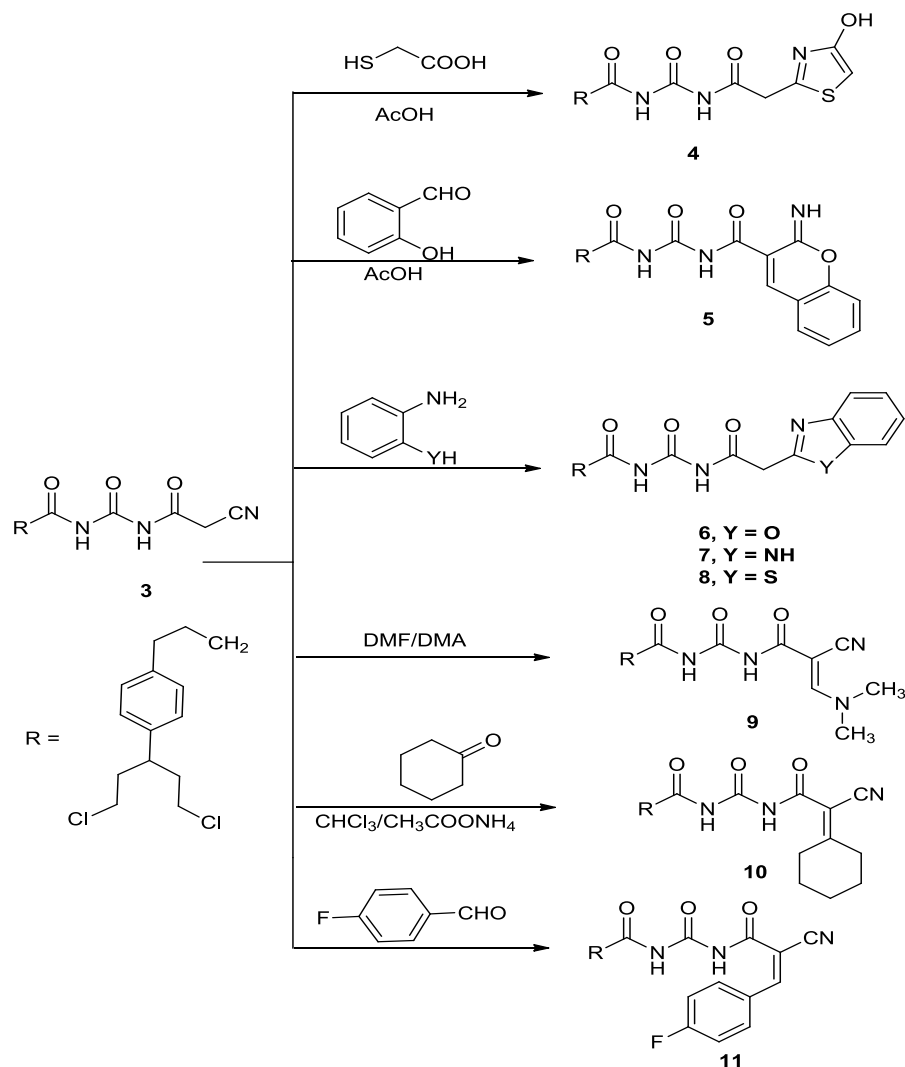
Structure Activity Relationship

The high activity of compound **4**, the most active compound among the series, may be attributed to the presence of the hydroxyl thiazole, which could be sterically favored causing a good binding and fitting with the receptor. The activity of compound **8** may be due to the presence of thiazole. The presence of sulphur atom in the heterocyclic ring increases activity. Compounds **2**, **5** and **7** are of moderate activity. The activity of compound **x** is weak due to the absence of thiazole ring.

CONCLUSION

New compounds (**x-3**) have been synthesized by the reaction of chlorambucil with thionyl chloride to give (**x**). Compound **x** reacted with thiocarbohydrazide to yield compound (**1**) which was cyclized by refluxing with acetic anhydride to give compounds (**2**), also compound (**x**) was reacted with cyanoacetylurea to form compound (**3**). New compounds (**4-11**) have been synthesized by the reaction of compound (**3**) with thioglycolic acid and/or salisaldehyde to produce compounds (**4**, **5** respectively). Also compound **3** was reacted with *o*-amino phenol, *o*-phenylene diamine and/or *o*-amino thiophenol to give compounds **6-8**, while compound **9** was obtained when compound **3** was reacted with DMF-DMA.

Compounds **10,11** were obtained when compound **3** was reacted with cyclohexanone and *p*-flouro-benzaldehyde. The antitumor activity of new compounds has been evaluated by studying their cytotoxicity against breast cancer cell line MCF7. The tested compounds **2, 5, 7** against breast cancer MCF7 cell line, was showed moderated activity and compound **8** showed activity against breast cancer MCF7 cell line, while compound **4** showed higher activity than doxorubicin.



Scheme 2

Experimental:

Solid compounds were recrystallized to constant melting points and dried in vacuum in drying pistol containing sodium hydroxide and P_2O_5 . All melting points are uncorrected were taken in open capillaries on a Gallen Kamp Apparatus. Microanalyses were carried out at the Micro-Analytical Unit National Research Centre and Faculty of Science, Cairo University. IR spectra were carried out on FT/IR 300 E Jasco using KBr discs. 1H NMR spectra were measured in $DMSO-d_6$ using Joel EX.270NMR spectrometer. The mass spectra were recorded on Finnigan SSQ7000 spectrometer.

General procedure for preparation of compound (x)

A mixture of 4-(4-(bis(2-chloroethyl)amino)phenyl)butanoic acid (5mmol) and thionyl chloride (10mmol) in 50 ml, dry benzene was refluxed for 8 hours. The reaction mixture was followed by TLC evaporate solvent using rotatory, washed several times with benzene. A solid that formed was filtered off, dried and recrystallized from ethyl acetate.

4-(4-(bis(2-chloroethyl)amino)phenyl)butanoyl chloride(x):

Yield 40% black powder, mp, 197-200°C, IR (KBr, cm⁻¹): 3045(CH-aromatic), 2895(CH-aliphatic), 1670 (C=O); ¹HNMR (DMSO-d⁶): δ 1.67 (m, 2H, -CH₂), 2.46 (t, 2H, CH₂CO), 2.74 (t, 2H, CH₂-Ar), 3.63(m, 4H, 2CH₂Cl), 3.68(m, 4H, 2CH₂N), 6.87-6.94 (m, 4H, Ar-H). MS *m/z* 322.5 (M⁺, 52%). Anal. calc. For [C₁₄H₁₈Cl₃NO]: C, 52.09; H, 5.58; N, 4.34; Found: C; 52.07; H, 5.57; N, 4.32.

General procedure for preparation of compound (1)

A solution of compound **x** (5mmol) and thiocarbonylhydrazide (5mmol) in 20 ml ethanol and few drops of triethylamine were heated under reflux for 12h. The reaction is monitoring by TLC. A solid product that precipitated after concentration was filtered off, washed with petroleum ether, dried and recrystallized from ethanol.

(4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(hydrazinecarbonothionyl)butanehydrazide (1):

Yield 41%, dark brown powder, mp. 111-113°C, IR (KBr, cm⁻¹): 3400-3200 (3NH, NH₂), 1699(C=O), 1219(C=S); ¹HNMR (DMSO-d₆): δ 1.95 (m, 2H, -CH₂), 2.30 (t, 2H, CH₂ CO), 2.58 (t, 2H, CH₂-Ar), 3.65 (m, 4H, 2CH₂Cl) 3.85 (m, 4H, 2CH₂-N), 5.20 (d, 2H, NH₂, D₂O exchangeable), 6.75-6.83 (m, 4H, Ar-H), 8.10 (t, 1H, NH, D₂O exchangeable), 9.25 (br_s, 1H, 1NH, D₂O exchangeable), MS: *m/z* 392 (M⁺, 24%). Anal. Calc. For [C₁₅H₂₃Cl₂N₅OS]: C, 45.91; H, 5.86; N, 17.85. Found: C, 45.90; H, 5.84; N, 17.83.

2-acetyl-3-(4-(4-(bis(2-chloroethyl)amino)phenyl)butanoyl)tetrazolidine-5-thione (2):

A solution of compound **1** (5mmol) and acetic anhydride (15ml) was heated under reflux at 90°C for 10h. The reaction is monitoring by TLC. A solid was precipitated after pouring onto cooled water, filtered off, washed with water, dried and recrystallized from ethanol.

Yield 45% brown powder, mp. 223-225°C IR (KBr, cm⁻¹) 3200-3190 (2NH), 1699, 1690 (2C=O), 1192 (C=S); ¹HNMR (DMSO-d₆): δ 1.90 (m, 2H, -CH₂-), 2.00(s, 3H, CH₃), 2.30 (t, 2H, CH₂ CO), 2.60(t, 2H, CH₂-Ar), 3.64(m, 4H, 2CH₂-Cl), 3.67 (m, 4H, 2CH₂-N), 6.77-6.85 (m, 4H, Ar-H), 8.40 (br_s, 1H, NH, D₂O exchangeable), 9.20 (br_s, 1H, NH, D₂O exchangeable). MS *m/z* 432 (M⁺, 31%). Anal. Calc. For [C₁₇H₂₃Cl₂N₅O₂S]: C, 47.22; H, 5.32; N, 16.20. Found: C, 47.23; H, 5.30; N, 16.19.

4-4-(bis(2-chloroethyl)amino)phenyl-N-[(2-cyanoacetylcarbamoyl)butanamide (3):

A mixture of compound (**x**) (5mmol) and cyanoacetyl urea (5mmol) in 20 ml absolute alcohol; and 1ml of triethyl amine was refluxed for 5h. A solid was precipitated after cooling, was filtered off, and washed with petroleum ether, dried and recrystallized from ethanol. Yield 40% brown powder, mp, 148-150°C IR (KBr, cm⁻¹): 3200-3100(2NH), 2255(CN), 1705, 1686, 1670 (3C=O); ¹HNMR (DMSO-d₆): δ 1.72 (m, 2H, -CH₂), 2.45 (t, 2H, CH₂CO), 2.74 (t, 2H, CH₂ Ar), 3.64 (m, 4H, 2CH₂-Cl), 3.69(m, 4H, 2CH₂ N), 4.30 (s, 2H, CH₂ CN), 6.79-6.89 (m, 4H, Ar-H), 9.70 (br_s, 2H, 2NH, D₂O exchangeable). MS *m/z* 414 (M⁺+1 22%). Anal. Calc. For [C₁₈H₂₂Cl₂N₄O₃]: C, 52.30; H, 5.32; N, 13.55. Found: C, 52.29; H, 5.30; N, 13.53.

(4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(2-(4-hydroxythiazol-2-yl)acetylcarbamoyl) butan amide (4):

A solution of compound (**3**) (5mmol) and thioglycollic acid (5mmol) in 15ml glacial acetic acid was heated under refluxing for 10h. The reaction followed by TLC. The reaction was cooled and poured onto ice cooled water. The precipitate was filtered off, washed with water, dried and recrystallized from acetic acid.

Yield 47% brown powder, mp., 185-187°C IR (KBr, cm⁻¹): 3400(OH), 3150-3120 (2NH), 1695; 1680, 1665 (3C=O), 1600 C=C; ¹HNMR (DMSO-d₆): δ 1.75 (m, 2H, -CH₂-), 2.46 (t, 2H, CH₂ CO), 2.74 (t, 2H, CH₂Ar), 3.62 (m, 4H, 2CH₂-Cl), 3.68 (m, 4H, 2CH₂-N), 4.40 (s, 2H, CH₂), 6.75-6.85 (m, 4H, Ar-H), 7.23 (s, 1H, =CH, thiazole), 9.20 (br_s, 2H, NH, D₂O exchangeable), 12.10 (s, 1H, OH, D₂O exchangeable). MS *m/z* 487 (M⁺, 37%). Anal. Calc. For [C₂₀H₂₄Cl₂N₄O₄S]: C, 49.28; H, 4.92; N, 11.49. Found: C, 49.30; H, 4.91; N, 11.47.

N-(4-(4-(bis(2-chloroethyl) amino) phenyl) butanoyl carbamoyl)-2-imino-2H-chromene-3-carboxamide (5):

A solution of compound **3** (5mmol) and salisaldehyde (5mmol) in 15ml glacial acetic acid was refluxed for 8h. The solid that precipitated after cooling was filtered off, washed with water, dried and recrystallized from acetic acid.

Yield 47% brown powder, mp. 240-242°C IR(KBr, cm^{-1}) 3190-3170 (3NH), 1700, 1681, 1670 (3C=O); ^1H NMR (DMSO- d_6): δ 1.73 (m, 2H, -CH₂-), 2.46 (t, 2H, CH₂CO), 2.76 (t, 2H, CH₂Ar), 3.65 (m, 4H, 2CH₂-Cl), 3.68 (m, 4H, 2CH₂-N), 6.77-6.86 (m, 4H, Ar-H), 7.01-7.10 (m, 4H, Ar-H), 7.90 (br_s, 2H, 2NH, D₂O exchangeable), 8.53 (s, 1H, chromene), 13.00 (br_s, 1H, =NH). MS m/z 518 (M^+ +1, 17%). Anal. Calc. For [C₂₅H₂₆Cl₂N₄O₄]: C, 58.02; H, 5.02; N, 10.83; Found: C, 58.00; H, 5.00; N, 10.84.

General procedure for preparation of compounds (6-8):

A mixture of compound **3** (5mmol), *o*-amino phenol, *o*-phenylene diamine and/ or *o*-amino thiophenol (5mmol) in 20ml ethenol in the presence of drops of piperidine was refluxed for 10h. The reaction is monitoring by TLC. A solid that precipitated after pouring onto ice cold water was filtered off, washed with water, dried and finally recrystallized from ethanol.

N-(4-(4-(bis(2-chloroethyl)amino) phenyl) butanoyl) carbamoyl) (benzo[d]oxazole-carboxamide (6):

Yield 41% orange powder, mp. 281-283 °C, IR(KBr, cm^{-1}): 3125-3110 (2NH), 1694, 1683, 1670 (3C=O) 1654(C=N); ^1H NMR (DMSO- d_6): δ 1.91 (m, 2H, -CH₂-), 2.15 (t, 2H, CH₂CO), 2.35 (t, 2H, CH₂-Ar), 3.55 (m, 4H, 2CH₂-Cl), 3.62 (m, 4H, 2CH₂-N), 4.40 (s, 2H, CH₂), 6.72-6.82 (m, 4H, Ar-H), 7.19-7.26 (m, 4H, Ar-H of benzoxazole), 9.70 (br_s, 2H, 2NH, D₂O exchangeable). MS m/z 507 (M^+ +2 17%). Anal. Calc. For [C₂₄H₂₆Cl₂N₄O₄]: C, 57.02; H, 5.14; N, 11.08; Found: C, 57.00; H, 5.12; N, 11.09.

N-(4-(4-(bis(2-chloroethyl)amino) phenyl) butanoyl) carbamoyl) -1H-benzo [d] imidazole-2-carboxamide (7):

Yield 47% brown powder, mp. >300°C, IR(KBr, cm^{-1}): 3180-3100 (3NH), 1700, 1675, 1660 (3C=O), 1650(C=N); ^1H NMR (DMSO- d_6): δ 1.91 (m, 2H, -CH₂-), 2.17 (t, 2H, CH₂CO), 2.48 (t, 2H, CH₂Ar), 3.60 (m, 4H, 2CH₂-Cl), 3.64 (m, 4H, 2CH₂-N), 4.35(s, 2H, CH₂) 5.97 (s, 1H, NH, D₂O exchangeable), 6.80-6.90 (m, 4H, Ar-H), 7.20-7.24 (m, 2H, benzimidazol H), 7.81-7.85 (m, 2H, benzimidazol H), 9.90 (br_s, 2H, NH, D₂O exchangeable); MS m/z 504 (M^+ , 13%). Anal. Calc. For [C₂₄H₂₇Cl₂N₅O₃]: C, 57.14; H, 5.35; N, 13.88; Found: C, 57.12; H, 5.33; N, 13.86.

N-(4-(4-(bis(2-chloroethyl)butanoyl)[benzo][d]thiazole-2-carboxamide (8):

Yield 55%, black powder, mp. > 300°C, IR(KBr, cm^{-1}): 3065-3000 (2NH), 1710, 1695, 1680 (3C=O), 1648 (C=N); ^1H NMR (DMSO- d_6): δ 1.92 (m, 2H, CH₂-), 2.19 (t, 2H, CH₂CO), 2.58 (t, 2H, CH₂-Ar), 3.58 (m, 4H, 2CH₂Cl), 3.62 (m, 4H, 2CH₂N), 4.37(s, 2H, CH₂), 6.67-6.77 (m, 4H, Ar-H), 7.50-7.58 (m, 4H, Ar-H of benzothiazole), 9.80 (br_s, 2H, NH, D₂O exchangeable). MS m/z 523 (M^+ +2 17%). Anal. Calc. For [C₂₄H₂₆Cl₂N₄O₃S]: C, 55.27; H, 4.99; N, 10.74; Found: C, 55.25; H, 4.98; N, 10.72.

General procedure for preparation of compound (9):

A mixture of compound (3) (5mmol) and *N,N*-dimethyl formamide dimethyl acetal (5mmol) in 15 ml of dry toluene was refluxed for 7 hours. The reaction is monitoring by TLC. A solid that precipitated after concentration was filtered off, washed with petroleum ether and dried, recrystallized from ethyl acetate.

4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(2-cyano-3 (dimethylamino) acryloyl carbamoyl) butanamide (9):

Yield 47%, black powder, mp 261-263, IR (KBr, cm^{-1}) 3200-3190 (2NH), 2250(CN), 1690, 1680, 1671 (3C=O), 1600 (C=C); ^1H NMR (DMSO- d_6): δ = 1.75 (m, 2CH, CH₂), 2.47 (t, 2H, CH₂CO), 2.74 (t, 2H, CH₂Ar), 3.14 (s, 3H, CH₃), 3.22 (s, 3H, CH₃), 3.65 (m, 4H, 2CH₂-Cl), 3.69 (m, 4H, 2CH₂N), 6.81-6.90 (m, 4H, ArH), 7.40 (s, 1H, =CH),

7.90 (brs, 1H, NH, D₂O exchangeable), 9.50 (brs, 1H, NH, D₂O exchangeable), MS *m/z* 469 (M⁺+1, 28%) Anal. calc. For [C₂₁H₂₇Cl₂N₅O₃]: C, 53.82; H, 5.76; N, 14.95; Found; C, 53.83; H, 5.74; N, 14.93.

General procedure for preparation of compound (10):

A mixture of compound **3** (5mmol), cyclohexanone (5mmol) and ammonium acetate (7.5mmol) in 30 ml chloroform in presence of few drops of glacial acetic acid was refluxed using Dean-Stark apparatus for 7 hours. The reaction is monitoring by TLC. A solid that precipitated after pouring onto ice cooled water was filtered off, washed with water and dried. The solid formed recrystallized from ethanol.

4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(2-cyano-2-cyclohexylideneacetyl carbamoyl)butanamide (10):

Yield 43% brown powder, mp. 95-97°C, IR(KBr, cm⁻¹) : 3150, 3100 (2NH), 2255 (CN), 1700, 1685, 1670 (3C=O) 1600 (C=C), ¹HNMR (DMSO-d⁶) : δ 1.40-1.70 (m, 6H, 3CH₂ cyclohexan), 1.75 (m, 2H, -CH₂-), 2.20-2.45 (m, 4H, 2CH₂ cyclohexan), 2.47 (t, 2H, CH₂CO), 2.74 (t, 2H, CH₂Ar), 3.65 (m, 4H, 2CH₂-Cl), 3.69 (m, 4H, 2CH₂N), 6.86-6.94 (m, 4H, Ar-H), 8.90 (brs, 1H, 1NH, D₂O exchangeable), 9.20 (brs, 1H, 1NH, D₂O exchangeable). MS *m/z* 494 (M⁺+1, 41%). Anal. calc. For [C₂₄H₃₀Cl₂N₄O₃]: C, 58.41; H, 6.08, N, 11.35; Found : C,58.37; H, 6.05; N, 11.33.

General procedure for preparation of compound (11):

A mixture of compound **3** (5mmol) and *p*-fluorobenzaldehyde (5mmol) in absolute ethanol 30 ml in presence of few drops of glacial acetic acid was refluxed for 6 hours. The reaction is monitoring by TLC. A solid product that precipitated after concentration was filtered off, washed with water several times and dried. The solid recrystallized from ethanol.

4-(4-(bis(2-chloroethyl)amino)phenyl)-N-2-cyano-3-(4-Fluoro phenyl acryloyl carbamoyl) butanamide (11):

Yield 52% green powder, mp. >300, IR (KBr, cm⁻¹) : 3190, 3150 (2NH), 2253 (CN), 1690, 1683, 1655 (3C=O), 1600 (C=C); ¹HNMR (DMSO-d⁶) : δ 1.69 (m, 2H, -CH₂-), 2.45 (t, 2H, CH₂CO), 2.75 (t, 2H, CH₂-Ar), 3.62 (m, 4H, 2CH₂-Cl), 3.67 (m, 4H, 2CH₂N), 6.86-7.10 (m, 8H, Ar-H), 7.60 (s, 1H, =CH), 8.90 (brs, 1H, NH, D₂O exchangeable), 9.78 (brs, 1H, NH, D₂O exchangeable). MS *m/z* 520 (M⁺+1, 36%), Anal. calc. For [C₂₅H₂₅Cl₂FN₄O₃]: C, 57.80; H,4.81; N, 10.78; Found : C, 57.78; H, 4.79; N, 10.75.

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