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SYNTHESIS OF NEW AZOLE AND AZINE SYSTEMS BASED ON CHROMENO[3,4-*c*]PYRROLE-3,4-DIONE AND INVESTIGATION OF THEIR CYTOTOXIC ACTIVITY

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Abstract – A novel series of 9-substituted-chromeno[4',3':3,4]pyrrolo[2,1-*c*]-[1,2,4]triazole-6,7-diones (**4**), chromeno[4',3':3,4]pyrrolo[1,2-*d*]tetrazole-6,7-dione (**5**), 9-phenyl-6*H*- chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazine-6,7(11*H*)-dione (**8**), chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7,9(10*H*)-trione (**10**), (3,5-substituted-pyrazol-1-yl)chromeno[3,4-*c*]pyrrole-3,4-dione (**15**-**19**) and thiazole derivatives (**28**-**37**) were synthesized using 1-hydrazinylchromeno[3,4-*c*]-pyrrole-3,4-dione (**2**) as a precursor. The newly synthesized compounds were characterized and evaluated for their cytotoxic activity.

INTRODUCTION

Various natural and synthetic biologically active compounds are found to be nitrogen containing heterocycles and have attracted continuing interest of the researchers in the past few decades due to their diverse therapeutic values.[1-4] Among diverse classes of antimicrobial agents, azoles have very good anti-bacterial properties and nitroimidazoles are the most known among them. Other azoles such as triazole,[5, 6] benzimidazole,[7-10] and pyrazole[11] are potent anti-bacterial agents.

Triazoles, in particular 1,2,4-triazole nucleus have been inserted into a wide variety of therapeutically interesting drug candidates including anti-inflammatory drugs, CNS stimulants, anxiolytic, sedatives, anti-microbial agents,[12,13] and anti-fungal agents.[14,15] Pyrazolones are correlated with broad spectrum of biological activities including antifungal, anti-inflammatory, antihistaminic, analgesic, anti-bacterial and anti-tumor properties.[16,17] 1,3,4-Oxadiazoles are an important type of oxygen and nitrogen containing aromatic heterocyclic compounds, have likable electronic and charge-transport properties and the varied functional groups are easily introduced into the structurally rigid oxadiazole ring. These characteristics resulted in the broad potential applications of oxadiazole based derivatives in the field of medicinal chemistry.[18-20] Also, substituted benzimidazole derivatives have found commercial application in veterinarian medicine as anthelmintic agent and in various human therapeutic fields as antiulcer antihypertensive, antiviral, antifungal and anti-histaminic.[21] These also show affinity towards set of enzymes and proteins receptors, therefore medicinal chemists would certainly qualify them as “privileged sub-structures” for drug design.[22] In a search of novel therapeutic agents, versatile heterocyclic moieties were appended in the 1,2,4-triazine nucleus via the interaction between functionalized 1,2,4-triazines with various nucleophilic and electrophilic reagents.[23] In particular, 1,2,4-triazine motif is exploited for pharmacological activities, as herbicides and pesticides, as well as in dyes. In drug discovery, for examples, pyrrolo-triazines were found as potent PI3K inhibitors.[24] On the other hand, recently, the syntheses of 4*H*-chromene and its derivatives have attracted massive attention due to their biological and pharmacological activities.[25] The 4*H*-chromene derivatives show several pharmacological properties such as antitumour,[26] diuretic,[27] and anti-coagulant.[25] Among 4*H*-chromenes, 2-amino-4-aryl-4*H*-chromenes have been identified for causing apoptosis, thus, useful as anti-cancer agents.[28] Figure 1 shows some of the reported biologically active chromene, azole and azine derivatives. Thus, the aforesaid biological significance of azole, azine and chromene derivatives compelled us to continue working[29-42] on the synthesis and screening of new heterocyclic compounds containing *N*-heterocyclic systems resulting in more potent compounds.

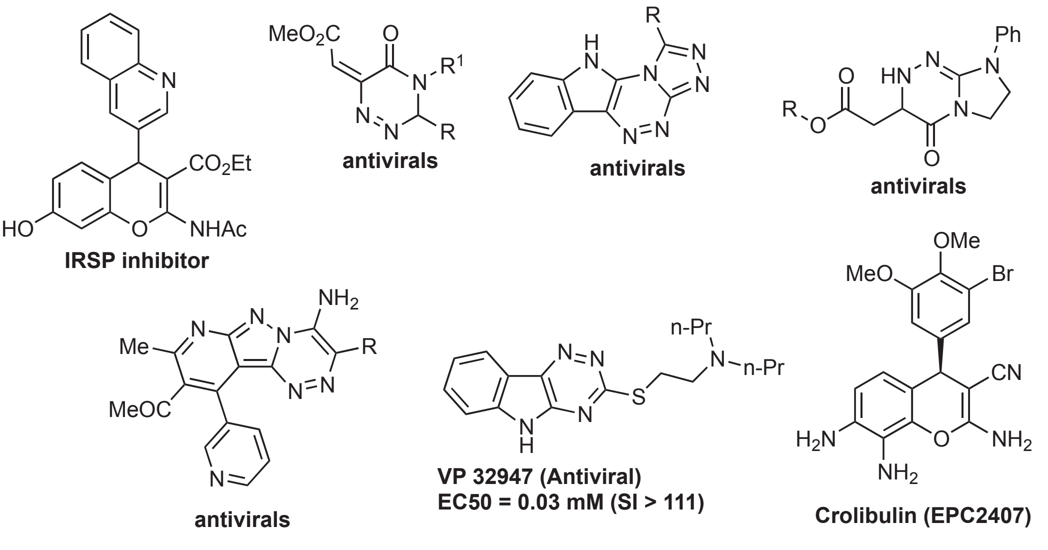
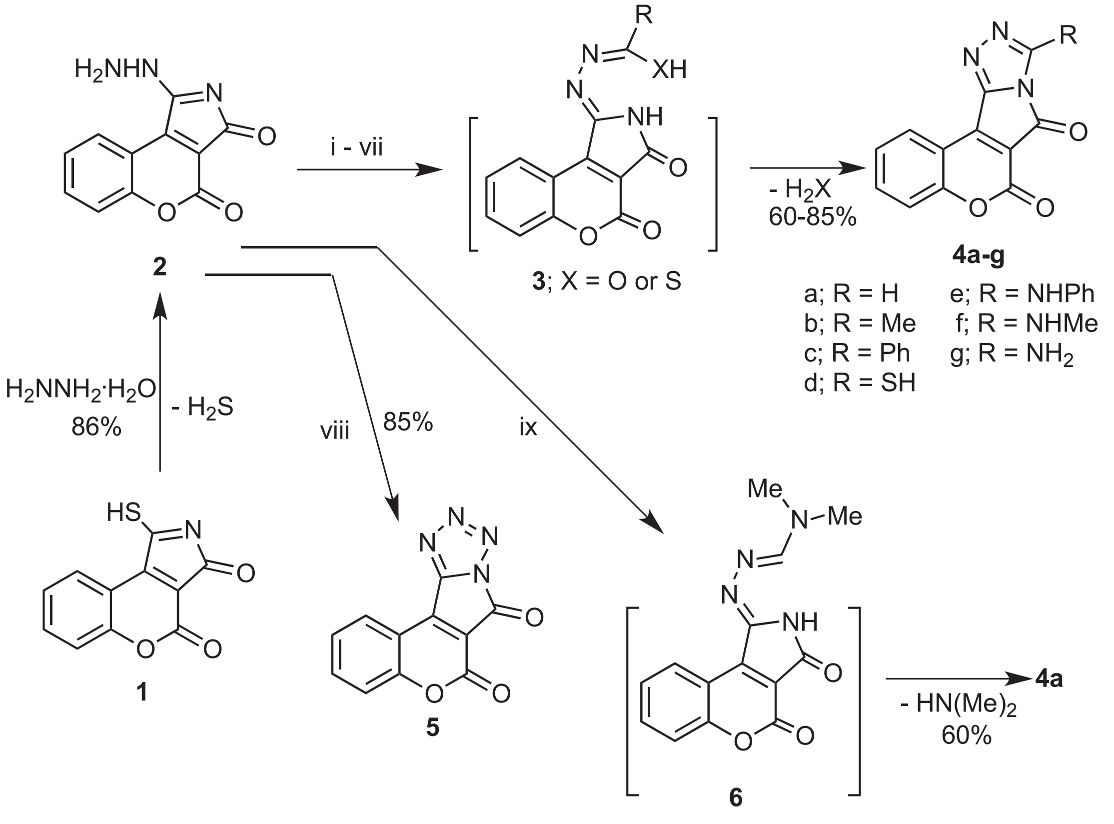


Figure 1. Representative examples of biologically active chromene, azole and azine derivatives

RESULTS AND DISCUSSION

The present work is an extension of our studies on chromene derivatives and their behavior as functional and bio-functional conjugates.[36-40] Herein we wish to report the synthesis of various types of functionalized chromene based *N*-heterocyclic derivatives and study their biological activities. Thus, heating under reflux 1-mercaptochromeno[3,4-*c*]pyrrole-3,4-dione (**1**)[40] with hydrazine hydrate in EtOH afforded 1-hydrazinylchromeno[3,4-*c*]pyrrole-3,4-dione (**2**) in 86% yield as shown in Scheme 1. The title compounds (**4** and **5**) were prepared by treating hydrazino derivative (**2**) with various one carbon donors, nitrous acid and dimethylformamide dimethyl acetal. The structures of isolated products **4** and **5** were evidenced by spectral data together with elemental analyses. For instance, the IR spectra of isolated products displayed in each case the absorption bands in the region 1665-1726 cm^{-1} due to the (C=O$_{str.}$) groups, while, neither the N-H nor the NH_2 groups were observed. The signals of the hydrazinyl protons originally observed in **2** (^{1}H NMR) at 5.42 and 8.51 ppm disappeared.

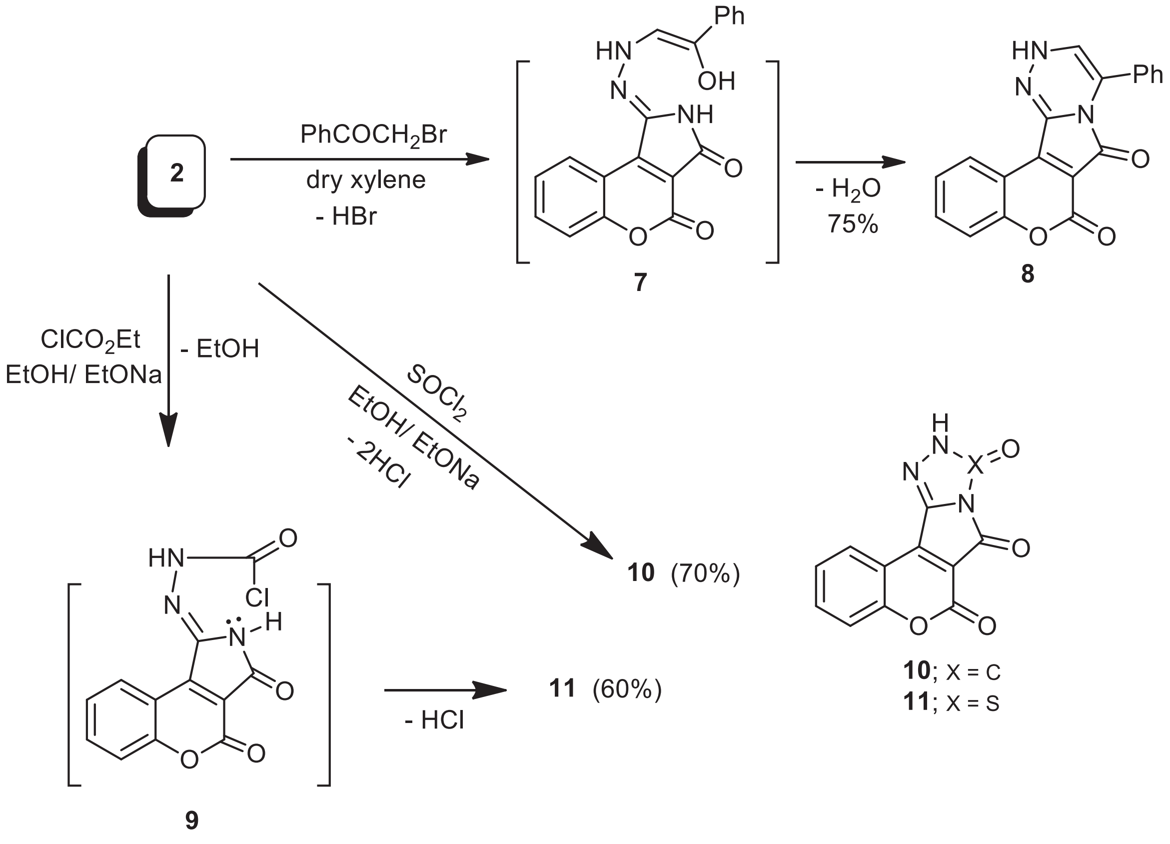


i = HCO_2H, ii = Ac_2O, iii = PhCOCl, iv = CS_2, v = PhNCS/ EtOH, vi = MeNCS/ EtOH,
vii = KSCN/ AcOH, viii = HONO, ix = DMF-DMA/ dry xylene

Scheme 1. Synthetic pathways for synthesis of pyrrolo[*c*][1,2,4]triazole derivatives **4a**-**g** and **5**

Hydrazinyl **2** underwent further, cyclization reactions. Thus, it was treated with phenacyl bromide under reflux in dry xylene to afford 9-phenyl-6*H*-chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]-triazine-6,7(11*H*)-dione (**8**) in 75% yield (**Scheme 2**). The structure of compound **8** was confirmed from its spectral data, where, its mass spectrum recorded molecular ion peak ($C_{19}H_{11}N_3O_3$) at *m/z* 329.08, while the IR spectra showed characteristic absorption bands at 3230, 1715, 1665 and 1620 cm^{-1} due to stretching of the N-H, two C=O, and C=N groups, respectively. Also, the ^1H NMR spectrum displayed a broad singlet at 11.89 ppm for the N-H group.

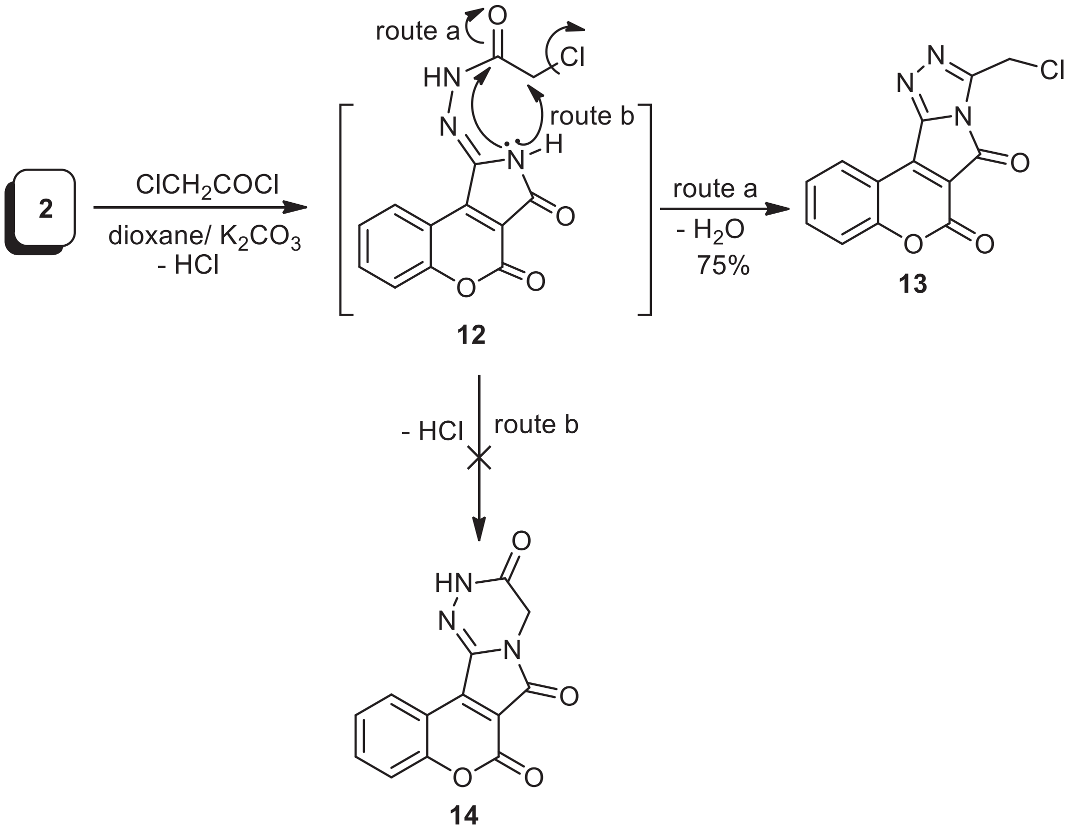
Likewise, cyclocondensation of hydrazinyl compound **2** with ethyl chloroformate and thionyl chloride in EtOH containing EtONa afforded chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7,9(10*H*)-trione (**10**) and 6*H*-chromeno[4',3':3,4]pyrrolo[2,1-*d*][1,2,3,5]thiatriazole-6,7,9(10*H*)-trione (**11**), respectively (**Scheme 2**). The mass spectrum of compound **10** showed a peak at *m/z* 255.03 corresponding to the molecular formula $C_{12}H_5N_3O_4$, while the IR spectrum showed strong stretching vibration bands at 3235, 1715, 1705, 1665 and 1620 cm^{-1} due to N-H*str*., three C=O*str*., and C=N*str*., respectively.



Scheme 2. Synthetic pathways of compounds **8-11**

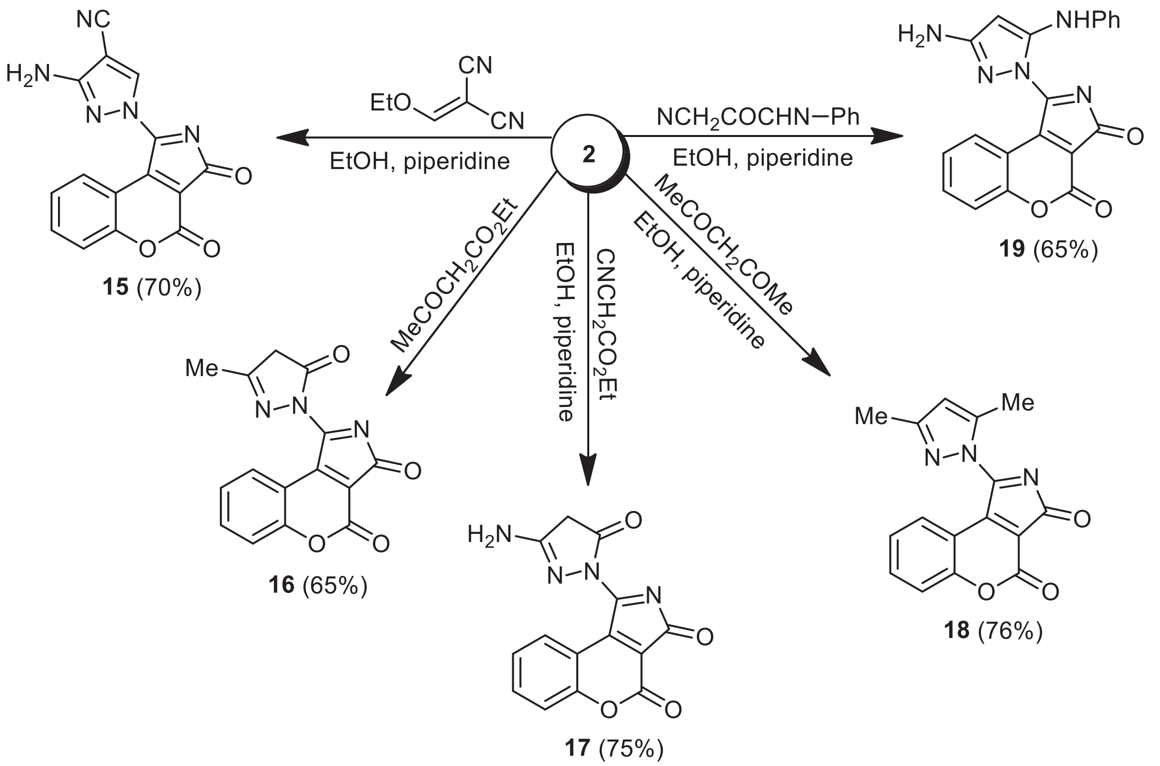
Further, investigation of the reactivity of the hydrazinyl moiety to annulate pyrrolo[2,1-*c*][1,2,4]triazole derivative was prepared. Thus, hydrazinyl **2** was treated with chloroacetyl chloride and anhydrous K_2CO_3 in warm dioxane to afford 9-(chloromethyl)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (**13**) in 75% yield (**Scheme 3**). The recorded mass at *m/z* 287.01 corresponding to the formula

$C_{13}H_6ClN_3O_3$, besides the two $C{=}O_{str}$ bands at ≈ 1665 and 1715 cm^{-1} and methylene singlet (^1H NMR) at 5.01 ppm all supported structure **13**.



Scheme 3. Synthesis of pyrrolo[2,1-*c*][1,2,4]triazole derivative **13**

The isolated pyrazoles (**15-19**) were obtained in fair to good yields *via* the cyclization of hydrazinyl **2** with various three carbon donors such as 2-(ethoxymethylene)malononitrile, ethyl acetoacetate, ethyl cyanoacetate, acetylacetone and 2-cyano-*N*-phenylacetamide, in ethanolic piperidine solution at reflux, (**Scheme 4**). The structures of compounds (**15-19**) were established on the basis of their elemental analysis and spectral data (IR, ^1H NMR, and MS). For example, the IR spectrum of **15** showed characteristic absorption bands at 3316, 2219, 1715 and 1665 cm^{-1} due to $NH_{2str.}$, $C{\equiv}N_{str.}$ and two $C{=}O_{str.}$, groups, respectively. Its mass spectrum showed the molecular ion at *m/z* 305.05 corresponding to its molecular formula $C_{15}H_7N_5O_3$. The ^1H NMR spectrum of **15** displayed a broad singlet at 6.83 (br s, NH_2, D_2O exchangeable) and 8.21 (s, 1H, pyrazolyl proton).

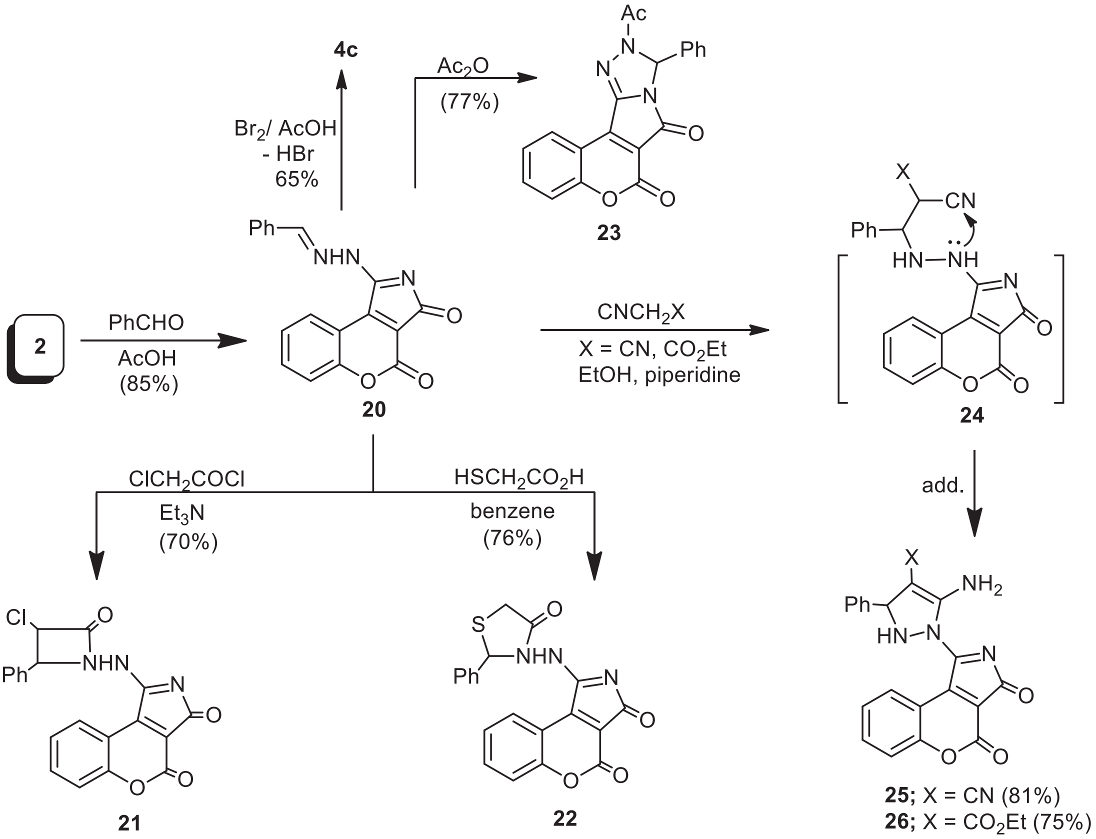


Scheme 4. Synthesis of pyrazole drevativies **15-19**

Then, compound **2** was incorporated in a set of investigations aiming at exploiting the reactivity of its hydrazinyl tag to build up the target azoles. Thus, compound **2** was treated with benzaldehyde in boiling glacial AcOH to afford 1-(2-benzylidenehydrazinyl)chromeno[3,4-*c*]pyrrole-3,4-dione (**20**) in 75% yield (**Scheme 5**). Compound **20** served as a key intermediate to attain the target azoles, thus, addition of chloroacetyl chloride to hydrazone **20** afforded the *β*-lactam derivative **21**. The structure of **21** was confirmed on the basis of its elemental analysis and spectral data. Its IR spectrum showed the presence of absorption bands at 3296, 1728, 1715, 1665 and 1627 cm^{-1} due to NH$_{2str.}$, three C=O$_{str.}$, and C=N$_{str.}$ groups, respectively. Its mass spectrum showed *m/z* at 393.05 (M$^+$, 35%) corresponding to a molecular formula $C_{20}H_{12}ClN_3O_4$. The ^{1}H-NMR spectrum of **21** showed two doublet signals at 4.46 and 5.21 ppm for the protons at C3 and C4 of the azetidine ring in addition to the expected protons. Also, compound **20** reacted with thioglycolic acid in dry benezene under reflux to afford thiazolidinone derivative **22**. The most characteristic signals of compound **22** in ^{1}H NMR spectrum belong to thiazolidinone methylene protons located at 3.83 ppm and to the exchangeable (NH) proton at 11.75 ppm.

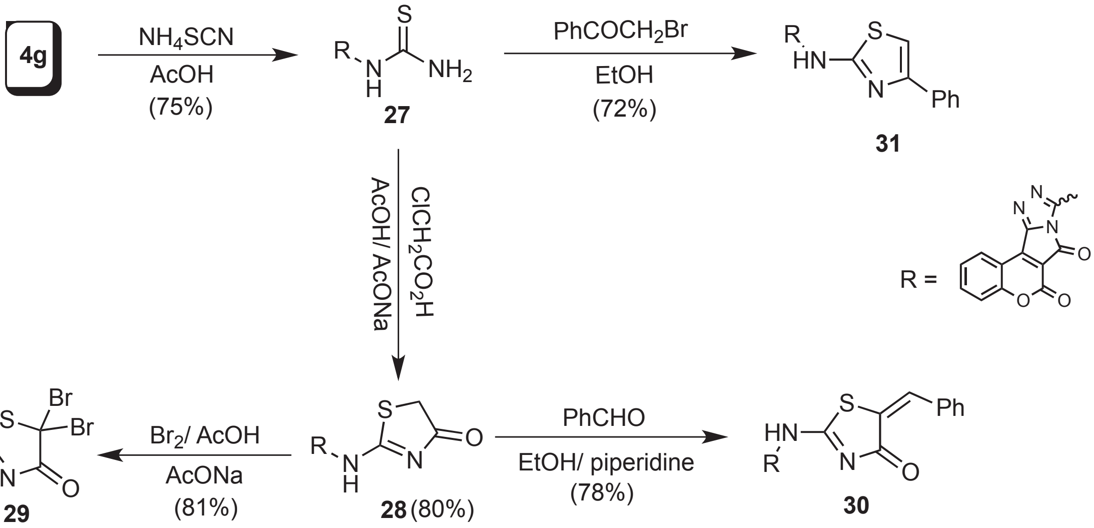
In another investigation compound **20** was cyclized into the pyrrolo-triazole **4c** *via* treating with bromine in AcOH acid at rt,[43] and compound **20** was cyclized to 10-acetyl-9-phenyl-9,10-dihydrochromeno-[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (**23**), upon treating with acetic anhydride.

Moreover, the benzylidenehydrazinyl **20** was cyclized to pyrazole derivatives **25** and **26** by treatment with certain active methylene reagents, for instance, malononitrile or ethyl cyanoacetate in refluxing EtOH containing a catalytic amount of piperidine. Formation of pyrazoles **25** and **26** is assumed to occur *via* the initial Michael addition reaction of the active methylene to the activated double bond in the benzylidenehydrazinyl **20** to give the intermediate **24**, which underwent intramolecular cyclization *via* nucleophilic addition of N-H to nitrile group to afford the final isolated products **25** and **26** (**Scheme 5**). The mass spectrum of **25** showed a molecular ion peak at *m/z* 383.10 corresponding to the molecular formula $C_{21}H_{13}N_5O_3$. Its IR spectrum showed intense absorption bands at 1620, 1665, 1715, 2225, 3235-3480 cm^{-1} due to $C{=}N_{str.}$, two $C{=}O_{str.}$, $C{\equiv}N_{str.}$, $NH_{str.}$ and $NH_{2str.}$ groups, respectively, besides the originally observed bands due to the pyrrole-chromene carbonyls, while The ^{1}H NMR spectrum displayed two D_2O-exchangeable broad singlets at 6.73 and 11.42 ppm for the NH_2 and NH groups, respectively.



Scheme 5. Reactions of hydrazone **20** with some nucleophiles

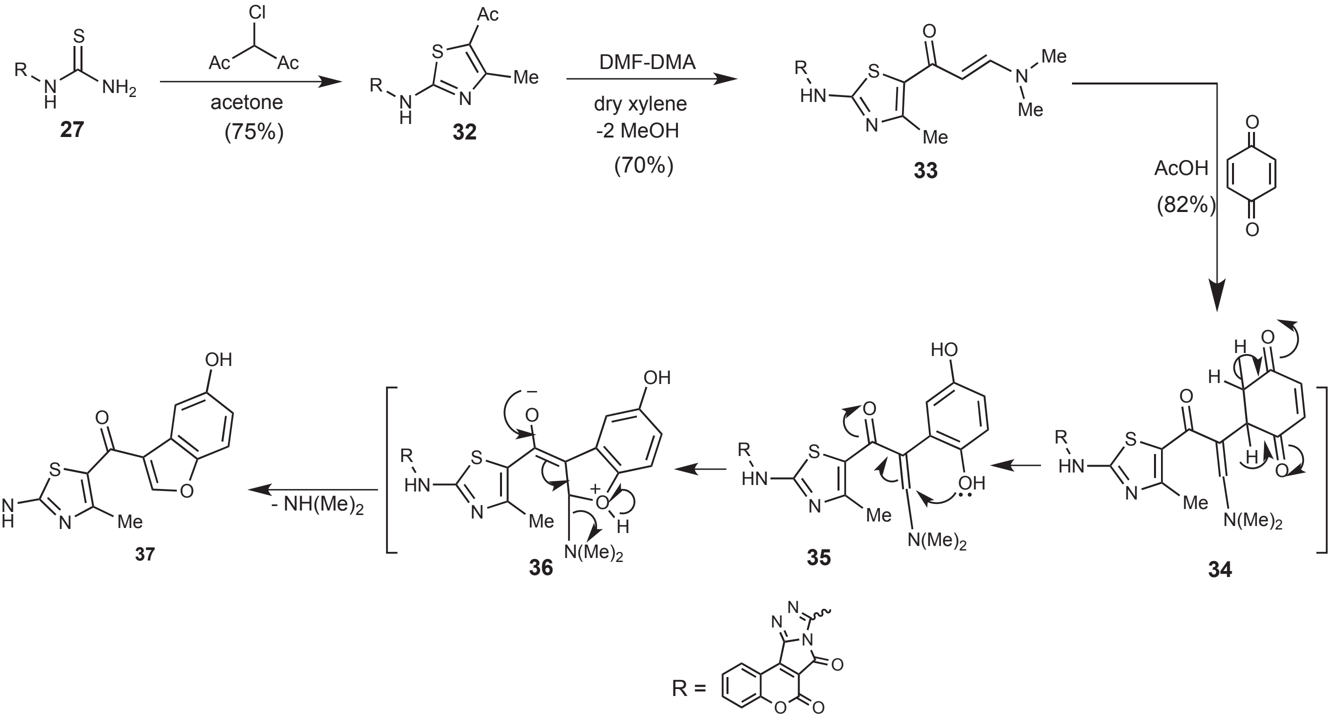
On the other hand, 9-aminochromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (**4g**) was treated with NH_4SCN in AcOH to afford **27** in 75% yield. The thioureido **27** was incorporated in a set of manipulations aiming at exploiting the reactivity of its thioureido tag, to build up the target thiazole architectures. Thus, compound **27** was treated with some α-halocarbonyl reagents using Hantzsch method to afford the target thiazole derivatives **28-36**[42,44] (**Scheme 6**). The thioureido **27** was converted to the thiazolone **28** *via* cyclization with chloroacetic acid, the spectrum of **28** (IR) declared the lack of C=S stretching band, while it showed presence of N–H stretching band at 3242 cm^{-1} and three C=O stretching bands at 1665, 1704 and 1715 cm^{-1}, besides the C=N stretching bands around 1610-1620 region. Its mass spectrum showed the molecular ion at *m/z* 353.02 corresponding to its molecular formula $C_{15}H_7N_5O_4S$. The ^{1}H NMR of **28** exhibited a singlet at 3.91 ppm attributed to the methylene protons of thiazolone moiety. Bromination of compound **28**, performed in AcOH at rt, yielded 9-((5,5-dibromo-4-oxo-4,5-dihydrothiophen-2-yl)amino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (**29**), and the active methylene group in the thiazolone moiety easily condensed with benzaldehyde to afford benzylidene derivative **30** in yield 78% (**Scheme 6**). Then, compound **27** was conveniently cyclized in ethanolic solution to afford 9-((4-phenylthiazol-2-yl)amino)chromeno[4',3':3,4]-pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (**31**), *via* treating with phenacyl bromide. The structure of compound **31** was confirmed based on its elemental and spectral data. Its IR spectrum confirmed the presence of intense absorption bands at 1610-1620, 1665, 1715 and 3276 cm^{-1} attributed to three C=N$_{str.}$, two C=O$_{str.}$, and N-H$_{str.}$ groups, respectively. The mass spectrum showed a peak at *m/z* 413.06 corresponding to the molecular formula $C_{21}H_{11}N_5O_3S$. Its ^{1}H NMR spectrum reveled a singlet at 6.86 ppm attributed to the thiazole-H5 proton.



Scheme 6. Synthesis of thiazole derivatives **28- 31**

Moreover, compound **27** on treating with 3-chloropentane-2,4-dione in refluxing acetone afforded 2-substituted 4-methyl-5-acetylthiazole derivative **32** in yield 75%, which on treating with dimethyformamide dimethyl acetal (DMF-DMA), in dry xylene, under reflux afforded the active enaminone **33** in 70% yield (**Scheme 7**). The IR spectrum of **33** revealed the presence of N–H stretching band at 3219 cm^{-1} and three C=O stretching bands at 1665, 1715 and 1721 cm^{-1}. Its mass spectrum showed the molecular ion at *m/z* 448.10 corresponding to its molecular formula $C_{21}H_{16}N_6O_4S$. The ^{1}H NMR spectrum exhibited two sharp singlets at 3.21 and 3.22 ppm assignable to *N,N*-dimethylamino protons, up filed doublet signal due to olefinic proton at *δ* 5.61 in addition to down field doublet signal due to the other olefinic proton at 8.72 ppm.

Finally, treatment of enaminone **33** with *p*-benzoquinone in AcOH at rt, afforded benzo[*b*]furan derivative **37**. Its IR spectrum showed broad band of the O-H$_{str.}$ group at 3432 cm^{-1}. While the mass spectrum showed a peak corresponding to the molecular ion at *m/z* 511.06, the ^{1}H NMR spectrum of **37** showed characteristic down field singlet at *δ* 8.71 of furan proton and revealed D$_2$O-exchangeable broad singlet at 9.62 and 11.25 ppm due to hydroxyl and N-H groups, respectively. Compound **37** is suggested to be formed *via* an initial addition of the electron-rich moiety C2 of the enaminone to the activated electron-poor double bond system of the quinone to form the intermediate **34**, which readily aromatized, cyclized, and eliminate dimethylamine moiety to afford benzo[*b*]furan derivative **37** (**Scheme 7**).



Scheme 7. Synthesis of benzo[*b*]furan derivative **36**

PHARMACOLOGICAL EVALUATION

CYTOTOXIC IMPACT

Nine compounds **2**, **5**, **8**, **15**, **19**, **20**, **21**, **22** and **25**, were selected and screened for their general *in vitro* cytotoxicity against two tumor cell lines; human breast cancer cell line (MCF-7) and human cervical cancer cell line (HeLa). The cytotoxicity of the compounds was determined by calculating their IC$_{50}$ values, which is the concentrations that caused a 50% reduction in cell growth compared to untreated control cells. Adriamycin (doxorubicin) was used as positive control for the comparison of cytotoxicity of synthesized compounds. Assays were performed in triplicate in three independent experiments and their mean values are presented in (**Table 1**). It is worth noting that the majority of the tested compounds possess significant cytotoxic activity towards the tested tumor cell lines. Also, the data represented in **Table 1** declared that the tested compounds showed greater potent cytotoxic potency against HeLa than MCF-7. The highest activities were observed for compounds **21**, **22**, **19**, **15**, **25**, **20** and **2** with $IC_{50} < 10.0$ μg/mL against HeLa and $IC_{50} < 20.0$ μg/mL against MCF-7. It was found that compounds **5** and **8** showed the less potency against the tumor cell lines with $IC_{50} > 25.0$ μg/mL against HeLa and MCF-7. However, the obtained results were compared with Adriamycin which has cytotoxic activity at concentrations 0.15 and 0.13 μg/mL against Hela and MCF-7, respectively.

Table 1. IC_{50} (μg/mL) values for some of the synthetic compounds against tumor cell lines

Comps.	IC_{50} (μg/mL) HeLa	IC_{50} (μg/mL) MCF-7	Comps.	IC_{50} (μg/mL) HeLa	IC_{50} (μg/mL) MCF-7
2	8.21 ± 2.26	11.51 ± 2.91	**20**	5.21 ± 1.29	6.62 ± 0.48
5	30.32 ± 1.46	33.11 ± 1.22	**21**	0.98 ± 1.60	1.21 ± 0.76
8	25.41 ± 1.24	29.36 ± 1.32	**22**	1.42 ± 1.12	2.15 ± 0.89
15	2.85 ± 1.29	5.12 ± 0.48	**25**	4.75 ± 1.29	6.12 ± 0.48
19	2.61 ± 2.36	4.89 ± 1.65	**Adriamycin**[e]	0.15 ± 0.21	0.13 ± 0.19

IC_{50} concentration of drug that decreases the viability of the cells by 50% compared to non-treated control cells; Each value represents mean of three readings ± SD; HeLa: Human cervical cancer cell line; MCF-7: Human Breast cancer cell line; Adriamycin: Positive control compound.

STRUCTURE ACTIVITY RELATIONSHIP (SAR)

The results of the anti-tumor screening demonstrated some definite and interesting facts about the structure-activity relationship (SAR) of synthesized molecules. In most cases, dependence of activity profile on structural modifications of the molecule is clear and dramatic. Alterations in potency profile of our compounds are also directly attributed with the structural alterations. The important highlights of structure-activity relationship are summarized here.

- The presence of a basic skeleton 1-hydrazinylchromeno[3,4-*c*]pyrrole-3,4-dione is essential for the broad spectrum of cytotoxic activity towards the investigated cell lines.

- **Effect of the presence of *β*-lactam moiety:** Introducing the *β*-lactam to the hydrazinyl derivative raises the cytotoxicity of compound **21**, to be nearly as potent as the positive control doxorubicin with IC50 = 0.98 *μg*/mL as the most active compound among the screened series.
- **Effect of thiazolidinone moiety:** Similarly thiazolidinone moiety in compound **22** makes the molecule exhibits excellent potency against the tested tumor cell lines.
- **Effect of the presence of pyrazole moiety:** The high activity of compounds **19, 15** and **25** may be attributed to the presence of the pyrazole tag(s). Introducing electron withdrawing groups, nitrile and phenyl reduces the potency of pyrazole derivative **25** when compared with the other tested pyrazoles **19** and **15**.
- **Effect of hydrazinyl tag:** The presence of hydrazinyl tag makes the molecule exhibits a good cytotoxicity, that is may be attributed to the presence of the side chain $–NHNH_2$ which could be sterically desirable and causes a good binding with the receptor; which makes the molecule highly potent against the tumor cell lines.
- Transformation and cyclization of the hydrazinyl derivative to pyrrolo[2,1-*c*][1,2,4]triazines derivatives **8** and **5** reduces the cytotoxic activity towards the tumor cell lines.

CONCLUSIONS

In the present work, 1-hydrazinylchromeno[3,4-*c*]pyrrole-3,4-dione (**2**) was prepared as new two–pharmacophoric–motif key intermediate in fair yield. The reactivity of the terminal hydrazinyl as well as thioureido **27** moieties were exploited in a series of manipulations encompassing cyclocondensation for the synthesis of new three and/or four–pharmacophoric–motif probes. The preliminary *in vitro* cytotoxic study revealed that the majority of the tested compounds possess significant anti-tumor activity towards the tested tumor cell lines. Also, the data declared that the tested compounds showed greater potent anti-tumor potency against Hela more than MCF-7. The highest activities were observed for compounds **21**, **22**, **19**, **15**, **25**, **20** and **2**, in decreasing order, while compounds **5** and **8** showed the less potency.

EXPERIMENTAL

Reagents were purchased from Sigma Aldrich and used without further purification. Reaction progress was monitored by TLC on silica gel precoated F254 Merck plates. Spots were visualized by ultraviolet irradiation. Melting points were determined on a Gallenkamp electrothermal melting point apparatus and are uncorrected. IR spectra were recorded as potassium bromide discs using Bruker-Vector 22 FTIR Spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on a 300 MHz Bruker WP spectrometer using DMSO–d_6 as solvent, while, TMS was used as internal standard. Mass spectra were recorded on a Hewlett Packard MS−5988 spectrometer at 70 eV. Elemental analyses were carried out at the Micro-analytical Unit of Cairo University, Egypt.

1-Hydrazinylchromeno[3,4-*c*]pyrrole-3,4-dione (2). A mixture of compound **1**[40] (0.23 g, 1 mmol), hydrazine hydrate 99.9% (0.5 mL) and absolute EtOH (30 mL) was heated under reflux for 2 h, then cooled to room temperature, the precipitate was filtered, washed with H_2O and recrystallized from EtOH as yellow crystals (86%), mp 245−247 ºC; IR (KBr): (cm^{-1}) 1612 (C=N$_{str.}$), 1665, 1715 (2C=O$_{str.}$), 3256-3342 (N-H$_{str.}$ and NH$_{2.str.}$); ^{1}H NMR (300 MHz, DMSO−d_6): δ 5.42 (br, s, H, NH, D_2O-exchangeable), 7.31−7.58 (m, 4H, Ar-H), 8.51 (br, s, 2H, NH_2, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO−d_6): 111.1, 125.5, 127.3, 128.3, 129.2, 131.1, 150.2, 161.2 (8 C=C), 152.6 (C=N), 159.4, 167.2 (2C=O); MS (*m/z*, %): 229.05 (M$^+$, 35%). Anal. Calcd for $C_{11}H_7N_3O_3$ (229.19): C, 57.65; H, 3.08; N, 18.33%. Found: C, 57.35; H, 3.02; N, 18.10%.

Chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (4a). *Method A:* A mixture of compound **2** (0.22 g, 1 mmol), formic acid (5 mL), and catalytic amount of concentrated hydrochloric acid was heated under reflux for 5 h, the reaction mixture was allowed to cool to rt, and poured onto H_2O (50 mL), the resulted solid was filtered off, washed with MeOH, dried, and recrystallized using EtOH to afford **4a** (75%) as orange crystals.

Method B: A solution of **2** (0.22 g, 1 mmol) and DMF-DMA (0.22 mL, 2 mmol) in dry xylene (30 mL) was heated under reflux for 5 h. The solvent was then removed in *vacuo* and the solid so obtained was triturated with petroleum ether, collected by filtration and recrystallized from EtOH to yield **4a** (60%), mp 185−187 ºC; IR (KBr): (cm^{-1}) 1620-1625 (2C=N$_{str.}$), 1665, 1722 (2C=O$_{str.}$); ^{1}H NMR (300 MHz, DMSO−d_6): δ 7.35–7.82 (m, 4H, Ar-H), 8.82 (s, 1H, Triaz$_{(C3)}$-H); ^{13}C NMR (75 MHz, DMSO−d_6): 117.5, 120.2, 125.5, 126.9, 128.3, 129.4, 154.3, 172.7 (8 C=C), 143.5, 155.6 (2 C=N), 159.4, 190.2 (2 C=O); MS (*m/z*, %): 239.03 (M$^+$, 25%); Anal. Calcd for $C_{12}H_5N_3O_3$ (239.19): C, 60.26; H, 2.11; N, 17.57%. Found: C, 60.11; H, 2.05; N, 17.11%.

9-Methylchromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (4b). Compound **2** (0.22 g, 1 mmol), was dissolved in Ac_2O (15 mL) and stirred under reflux for 7 h, the reaction mixture was allowed to cool to rt, and poured onto H_2O (50 mL). The resulted solid was filtered off, washed with MeOH, dried, and recrystallized using EtOH to afford **4b** (82%) as buff solid, mp 135−137 ºC; IR (KBr): (cm^{-1}) 1620-1627 (2C=N$_{str.}$), 1665, 1725 (2C=O$_{str.}$); ^{1}H NMR (300 MHz, DMSO−d_6): δ 1.92 (s, 3H, CH_3), 7.35–7.82 (m, 4H, Ar-H); MS (*m/z*, %): 253.05 (M$^+$, 40%); Anal. Calcd for $C_{13}H_7N_3O_3$ (235.21): C, 61.66; H, 2.79; N, 16.59%. Found: C, 61.32; H, 2.45; N, 16.41%.

9-Phenylchromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (4c). *Method A:* A mixture of compound **2** (0.22 g, 1 mmol), and benzoyl chloride (15 mL) was heated at reflux for 4 h, the reaction mixture was allowed to cool to rt, the resulted solid was filtered off, washed with MeOH, dried, and recrystallized using EtOH to afford **4c** (82%).

Method B: It was prepared according to previously reported work;[42] a mixture of benzylidenehydrazinyl **20** (0.62 g, 2 mmol) and anhydrous AcONa (3.3 g, 4 mmol,) were stirred in glacial AcOH (50 mL), then 10% v/v solution of bromine in glacial AcOH (10 mL) was added in a small portion. The reaction mixture was stirred at 25 ºC for 1 h, and then poured onto ice H_2O (200 mL). The precipitate was filtered, washed with water, followed by aqueous $NaHSO_3$ solution (10%) (250 mL), and the resulted crud product was recrystallized from EtOH to afford **4c** in 65% yield as yellow crystals, mp 172–174 ºC; IR (KBr): (cm^{-1}) 1620-1625 (2C=N$_{str.}$), 1665, 1722 (2C=O$_{str.}$); ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.35–7.82 (m, 9H, Ar-H); MS (*m/z*, %): 315.06 (M$^+$, 25%); Anal. Calcd for $C_{18}H_9N_3O_3$ (315.28): C, 68.57; H, 2.88; N, 13.33%. Found: C, 68.25; H, 2.65; N, 13.21%.

9-Mercaptochromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (4d). A mixture of compound **2** (0.22 g, 1 mmol) and carbon disulfide (5 mL) in pyridine (20) was heated on water bath for 4 h. The solid product thus formed was filtered off, washed several times with H_2O and recrystallized from dioxane to yield **4d** (70%) as orange crystals, mp 270–272 ºC; IR (KBr): (cm^{-1}) 1615-1620 (2C=N$_{str.}$), 1665, 1723 (2C=O$_{str.}$), 2453 (H-S$_{str.}$); ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.35–7.82 (m, 4H, Ar-H), 11.32 (br. s, 1H, S–H); MS (*m/z*, %): 271.01 (M$^+$, 65%); Anal. Calcd for $C_{12}H_5N_3O_3S$ (271.25): C, 53.13; H, 1.86; N, 15.49%. Found: C, 53.01; H, 1.67; N, 15.31%.

General procedure for preparation of compounds (4e,f). A mixture of **2** (0.22 g, 1 mmol) and phenyl isothiocyanate (0.16 mL, 1 mmol) or methyl isothiocyanate (0.07 g, 1 mmol) in absolute EtOH (30 mL) containing anhydrous K_2CO_3 (0.14 g), was heated under reflux for 12 h, cooled then poured into ice-cold H_2O. The solid product formed was filtered, washed with H_2O, dried, and crystallized from proper solvent.

9-(Phenylamino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (4e). Pale yellow crystals (DMF/EtOH (2:1)), in 65% yield, mp 289–291 ºC; IR (KBr): (cm^{-1}) 1615-1620 (2 C=N$_{str.}$), 1665, 1723 (2 C=O$_{str.}$), 3289 (N-H$_{str.}$); ^{1}H NMR (300 MHz, DMSO–d_6): δ 6.85–7.82 (m, 9H, Ar-H), 11.19 (br, s, 1H, NH, D_2O-exchangeable); MS (*m/z*, %): 330.08 (M$^+$, 35%); Anal. Calcd for $C_{18}H_{10}N_4O_3$ (271.25): C, 65.45; H, 3.05; N, 16.96%. Found: C, 65.24; H, 2.88; N, 16.76%.

9-(Methylamino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (4f). Yellow crystals (DMF/EtOH (2:1)), in 60% yield, mp 278–280 ºC; IR (KBr): (cm^{-1}) 1615-1620 (2C=N$_{str.}$), 1665, 1723 (2C=O$_{str.}$), 3289 (N-H$_{str.}$); ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.35–7.82 (m, 4H, Ar-H), 10.87 (br, s, 1H, NH, D_2O-exchangeable); MS (*m/z*, %): 268.06 (M^+, 40%); Anal. Calcd for $C_{13}H_8N_4O_3$ (268.23): C, 58.21; H, 3.01; N, 20.89%. Found: C, 58.04; H, 2.92; N, 20.68%.

9-Aminochromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (4g). A mixture of compound **2** (0.22 g, 1 mmol), and potassium thiocyanate (0.48 g, 5 mmol) was heated at reflux for 8 h in glacial AcOH (20 mL), and the reaction mixture was allowed to cool to rt, then poured into H_2O. The precipitate formed was collected by filtration, dried and recrystallized from EtOH/dioxane (2:1) to afford **4g** (85%) as yellow crystals, mp 252–254 ºC; IR (KBr): (cm^{-1}) 1610-1620 (2C=N*str.*), 1665, 1726 (2C=O*str.*), 3436 (NH_2*str.*); ^1H NMR (300 MHz, DMSO–*d6*): δ 7.35–7.82 (m, 4H, Ar-H), 6.89 (br, s, 2H, NH_2, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO–*d6*): 117.5, 120.2, 125.5, 126.9, 128.3, 129.4, 154.3, 172.7 (8C=C), 143.6, 157.6 (2 C=N), 159.4, 190.2 (2C=O); MS (*m/z*, %): 254.04 (M^+, 55%); Anal. Calcd for $C_{12}H_6N_4O_3$ (254.20): C, 56.70; H, 2.38; N, 22.04%. Found: C, 56.55; H, 2.15; N, 21.91%.

Chromeno[4',3':3,4]pyrrolo[1,2-*d*]tetrazole-6,7-dione (5). To an ice-cooled solution of **2** (0.22 g, 1 mmol) in 30 mL (HCl/AcOH (1:1)), a solution of sodium nitrite (2.0 g) in water (10 mL) was added dropwise. The solution was stirred at rt for an additional 2 h, and the crude product obtained was filtered off and recrystallized from EtOH afforded **5** (85%) as yellow crystals, mp 165–167 ºC; IR (KBr): (cm^{-1}) 1620 (C=N*str.*), 1630 (N=N*str.*), 1665, 1726 (2C=O*str.*); ^1H NMR (300 MHz, DMSO–*d6*): δ 7.35–7.82 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, DMSO–*d6*): 117.5, 120.2, 125.5, 126.9, 128.3, 129.4, 154.3, 172.7 (8C=C), 146.6 (C=N), 159.4, 190.2 (2C=O); MS (*m/z*, %): 240.03 (M^+, 30%); Anal. Calcd for $C_{11}H_4N_4O_3$ (240.17): C, 55.01; H, 1.68; N, 23.33%. Found: C, 54.89; H, 1.45; N, 23.21%.

9-Phenyl-6*H*-chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazine-6,7(11*H*)-dione (8). To a solution of **2** (0.22 g, 1 mmol) in EtOH (20 mL), phenacyl bromide (0.19 g, 1 mmol) was added. The mixture was refluxed for 8 h (monitored by TLC), then left to cool. The solid product was filtered off, washed with MeOH and recrystallized from EtOH to afford **8** (75%) as yellow crystals, mp 216–218 ºC; IR (KBr): (cm^{-1}) 1620 (C=N*str*), 1665, 1715 (2 C=O*str.*), 3230 (N-H*str.*); ^1H NMR (300 MHz, DMSO–*d6*): δ 7.33–7.82 (m, 10H, 9Ar-H and Pyrimi.$_{(C6)}$-H), 11.89 (br, s, H, NH, D_2O-exchangeable); MS (*m/z*, %): 329.08 (M^+, 51%); Anal. Calcd for $C_{19}H_{11}N_3O_3$ (329.31): C, 69.30; H, 3.37; N, 12.76%. Found: C, 69.14; H, 3.21; N, 12.38%.

Chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7,9(10*H*)-trione (10). To a cold solution of **2** (0.22 g, 1 mmol) in pyridine (10 mL), ethyl chloroformate (2 mL) was added dropwise. The mixture was refluxed for 4 h, then allowed to cool and poured into H_2O. The solid thus obtained was filtered off and recrystallized from EtOH to afford **10** (70%) as yellow crystals, mp 294–296 ºC; IR (KBr): (cm^{-1}) 1620 (C=N*str.*), 1665, 1705, 1715 (3C=O*str.*), 3235 (N-H*str.*); ^1H NMR (300 MHz, DMSO–*d6*): δ 7.35–7.82 (m, 4H, Ar-H), 11.93 (br, s, H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO–*d6*): 111.1, 125.2, 125.5, 127.3, 128.3, 129.2, 150.1, 157.8 (8C=C), 143.5 (C=N), 153.2, 159.3, 166.4 (3C=O); MS (*m/z*, %): 255.03 (M^+, 45%); Anal. Calcd for $C_{12}H_5N_3O_4$ (255.19): C, 56.48; H, 1.97; N, 16.47%. Found: C, 56.21; H, 1.75; N, 16.23%.

6*H*-Chromeno[4',3':3,4]pyrrolo[2,1-*d*][1,2,3,5]thiatriazole-6,7,9(10*H*)-trione (11). A solution of compound **2** (0.22, 1 mmol) in EtOH (30 mL) was treated with EtONa (0.023 g, 1 mmol from sodium metal) and thionyl chloride (1 mmol). The mixture was heated on water bath for 8 h. The mixture was diluted with H_2O and extracted with AcOEt, the organic layers were dried over anhydrous sodium sulfate and the AcOEt was removed under reduced pressure and recrystallized from EtOH to afford **11** (60%) as yellow crystals. mp 174–176 ºC; IR (KBr): (cm^{-1}) 1620 ($C{=}N_{str.}$), 1665, 1715 ($C{=}O_{str.}$), 3235 ($N\text{-}H_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 7.35–7.82 (m, 4H, Ar-H), 11.85 (br, s, H, NH, D_2O-exchangeable); MS (*m/z*, %): 275.00 (M^+, 20%); Anal. Calcd for $C_{11}H_5N_3O_4S$ (275.24): C, 48.00; H, 1.83; N, 15.27%. Found: C, 47.86; H, 1.63; N, 15.03%.

9-(Chloromethyl)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (13). A mixture of **2** (0.22 g, 1 mmol) and chloroacetyl chloride (0.11 mL, 1 mmol) in dry dioxane (30 mL) in the presence of anhydrous K_2CO_3 (0.13 g, 1 mmol) was refluxed on a water bath for 12 h. The reaction mixture was evaporated under reduced pressure and the residue was triturated with H_2O. The separated solid was filtered off, washed with H_2O, dried and recrystallized afforded **13** (75%) as buff solid, mp 195–197 ºC; IR (KBr): (cm^{-1}) 1620-1627 ($2C{=}N_{str.}$), 1665, 1715 ($2C{=}O_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 5.01 (s, 2H, CH_2), 7.35–7.82 (m, 4H, Ar-H); MS (*m/z*, %): 287.01 (M^+, 40%), 289.01 (M+2, 13%); Anal. Calcd for $C_{13}H_6ClN_3O_3$ (287.66): C, 54.28; H, 2.10; Cl, 12.32; N, 14.61%. Found: C, 54.02; H, 2.05; Cl, 12.14; N, 14.41%.

General procedure for preparation of pyrazole derivatives 15-19. A mixture of **2** (0.22 g, 1 mmol) and some selected three carbon donors such as 2-(ethoxymethylene)malononitrile (0.12 g, 1 mmol), ethyl acetoacetate (0.13 mL, 1 mmol), ethyl 2-cyanoacetate (0.11 mL, 1 mmol), acetylacetone (0.10 mL, 1 mmol) and 2-cyano-*N*-phenylacetamide (0.16 mL, 1 mmol) in EtOH (30 mL) containing piperidine (0.5 mL) was refluxed for 5-8 h then left to cool at rt. The solid product was filtered off, washed with EtOH and recrystallized from EtOH to afford **15-19** in 65-76% yields.

1-(3-Amino-4-cyano-1*H*-pyrazol-1-yl)chromeno[3,4-*c*]pyrrole-3,4-dione (15). Yellow crystals (70%), mp 128–130 ºC; IR (KBr): (cm^{-1}) 1615, 1620 ($2C{=}N_{str.}$), 1665, 1715 ($2C{=}O_{str.}$), 2219 ($C{\equiv}N_{str.}$), 3316 ($N\text{-}H_{2str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 6.83 (br, s, 2H, NH_2, D_2O-exchangeable), 7.35–7.82 (m, 4H, Ar-H), 8.21 (s, H, Pyraz.$_{(C5)}$-H); ^{13}C NMR (75 MHz, DMSO–d_6): 111.1, 125.3, 127.3, 128.3, 129.2, 131.1, 131.2, 150.2, 161.1 (9C=C), 115.5 (C≡N), 155.3, 163.9 (2C=N), 159.3, 167.4 (2C=O); MS (*m/z*, %): 305.05 (M^+, 20%); Anal. Calcd for $C_{15}H_7N_5O_3$ (305.25): C, 59.02; H, 2.31; N, 22.94%. Found: C, 58.89; H, 2.18; N, 22.81%.

1-(3-Methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)chromeno[3,4-*c*]pyrrole-3,4-dione (16). Pale yellow crystals (65%), mp 185–187 ºC; IR (KBr): (cm^{-1}) 1615, 1620 (2C=$N_{str.}$), 1665, 1689, 1715 (3C=$O_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 3.15 (s, 3H, CH_3), 3.35 (s, 2H, CH_2), 7.45–7.84 (m, 4H, Ar-H); MS (*m/z*, %): 295.06 (M^+, 35%); Anal. Calcd for $C_{15}H_9N_3O_4$ (295.25): C, 61.02; H, 3.07; N, 14.23%. Found: C, 59.87; H, 2.98; N, 14.01%.

1-(3-Amino-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)chromeno[3,4-*c*]pyrrole-3,4-dione (17). Yellow crystals (75%), mp 231–233 ºC; IR (KBr): (cm^{-1}) 1615, 1620 (2C=$N_{str.}$), 1665, 1689, 1715 (3C=$O_{str.}$), 3425 (N-$H_{2str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 3.35 (s, 2H, CH_2), 6.53 (br, s, 2H, NH_2, D_2O-exchangeable), 7.45–7.84 (m, 4H, Ar-H); MS (*m/z*, %): 296.05 (M^+, 15%); Anal. Calcd for $C_{14}H_8N_4O_4$ (296.24): C, 56.76; H, 2.72; N, 18.91%. Found: C, 56.57; H, 2.58; N, 18.75%.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)chromeno[3,4-*c*]pyrrole-3,4-dione (18). Yellow crystals (76%), mp 158–160 ºC; IR (KBr): (cm^{-1}) 1615, 1620 (2C=$N_{str.}$), 1665, 1715 (2C=$O_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 3.01 (s, 3H, CH_3), 3.05 (s, 3H, CH_3), 6.15 (s, 1H, $Pyraz._{(C4)}$-H), 7.35–7.82 (m, 4H, Ar-H); MS (*m/z*, %): 293.08 (M^+, 20%); Anal. Calcd for $C_{16}H_{11}N_3O_3$ (293.28): C, 65.53; H, 3.78; N, 14.33%. Found: C, 65.21; H, 3.56; N, 14.13%.

1-(3-Amino-5-(phenylamino)-1*H*-pyrazol-1-yl)chromeno[3,4-*c*]pyrrole-3,4-dione (19). Brown crystals (65%), mp 227–229 ºC; IR (KBr): (cm^{-1}) 1615, 1620 (2C=$N_{str.}$), 1665, 1715 (2C=$O_{str.}$), 3425 (N-$H_{2str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 6.53 (br, s, 2H, NH_2, D_2O-exchangeable), 7.35–7.82 (m, 9H, Ar-H), 8.21 (s, H, $Pyraz._{(C5)}$-H), 12.53 (br, s, H, NH, D_2O-exchangeable); MS (*m/z*, %): 371.10 (M^+, 40%); Anal. Calcd for $C_{20}H_{13}N_5O_3$ (371.35): C, 64.69; H, 3.53; N, 18.86%. Found: C, 64.31; H, 3.15; N, 18.67%.

1-(2-Benzylidenehydrazinyl)chromeno[3,4-*c*]pyrrole-3,4-dione (20). A mixture of **2** (0.22 g, 1 mmol) and benzaldehyde (0.10 g, 1 mmol) in AcOH (20 mL) was refluxed for 5 h, the reaction mixture was allowed to cool to rt, the resulted solid was filtered off, washed with MeOH, dried, and recrystallized using EtOH to afford **20** (75%) as orange crystals, mp 269–271 ºC; IR (KBr): (cm^{-1}) 1620-1627 (2C=$N_{str.}$), 1665, 1715 (2C=$O_{str.}$), 3405 (N-$H_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 7.35–7.82 (m, 9H, Ar-H), 8.92 (s, 1H,-CH=N), 11.61 (br, s, H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO–d_6): 111.1, 125.5, 127.3, 128.3, 128.7, 129.1, 129.2, 131.0, 131.1, 133.6, 150.2, 161.2 (14C=C), 146.8, 152.6 (2C=N), 159.4, 167.2 (2C=O); MS (*m/z*, %): 317.08 (M^+, 50%); Anal. Calcd for $C_{18}H_{11}N_3O_3$ (317.30): C, 68.14; H, 3.49; N, 13.24%. Found: C, 68.05; H, 3.25; N, 13.11%.

1-((3-Chloro-2-oxo-4-phenylazetidin-1-yl)amino)chromeno[3,4-*c*]pyrrole-3,4-dione (21). To a well stirred solution of **20** (0.31 g, 1 mmol) and triethylamine (0.2 mL) in dry dioxane (20 mL), chloroacetyl chloride (0.11 mL, 1 mmol) was added dropwise at rt, then the reaction mixture was refluxed for 7 h. The precipitate of triethylamine hydrochloride was filtered and washed thoroughly with dioxane. The filtrate was evaporated to one-third of its original volume, cooled and poured into acidified ice/H_2O and the formed precipitate washed with H_2O thoroughly, dried and recrystallized from MeOH to afford **21** (70%) as brown powder, mp 263−265 ºC; IR (KBr): (cm^{-1}) 1627 (C=N$_{str.}$), 1665, 1715, 1728 (3C=O$_{str.}$), 3296 (N-H$_{str.}$); ^{1}H NMR (300 MHz, DMSO−d_6): δ 4.46 (d, *J* = 7.01 Hz, 1H, Azitid.$_{(C4)}$-H), 5.21 (d, *J* = 7.01 Hz, 1H, Azitid.$_{(C3)}$-H), 7.35–7.82 (m, 9H, Ar-H), 12.23 (br, s, H, NH, D_2O-exchangeable); MS (*m/z*, %): 393.05 (M$^+$, 35%), 395.05 (M+2, 12%); Anal. Calcd for $C_{20}H_{12}ClN_3O_4$ (393.78): C, 61.00; H, 3.07; Cl, 9.00; N, 10.67%. Found: C, 59.86; H, 2.92; Cl, 8.82; N, 10.42%.

1-((4-Oxo-2-phenylthiazolidin-3-yl)amino)chromeno[3,4-*c*]pyrrole-3,4-dione (22). An equimolar mixture of **20** (0.31 g, 1 mmol) and thioglycolic acid (0.14 mL, 1 mmol) in dry benzene (20 mL) was refluxed for 8 h, and the reaction mixture was evaporated to dryness under reduced pressure. The resulted precipitate filtered off, washed with ether and recrystallized from EtOH to afford **22** (76%) as yellow crystals, mp 275−277 ºC; IR (KBr): (cm^{-1}) 1627 (C=N$_{str.}$), 1665, 1715, 1730 (3C=O$_{str.}$), 3329 (N-H$_{str.}$); ^{1}H NMR (300 MHz, DMSO−d_6): δ 3.83 (s, 2H, Thiazoli.$_{(C2)}$-H), 5.23 (s, 1H, Thiazoli.$_{(C5)}$-H), 7.31–7.82 (m, 9H, Ar-H), 11.75 (br, s, H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO−d_6): 35.7 (CH_2−Thiazoli.), 65.1 (CH-Thiazoli.), 111.1, 125.3, 126.8, 127.1, 127.3, 128.3, 128.6, 129.2, 131.1, 139.2, 150.2, 161.1 (C=C), 152.5 (C=N), 159.3, 167.4 (2C=O); MS (*m/z*, %): 391.06 (M$^+$, 45%); Anal. Calcd for $C_{20}H_{13}N_3O_4S$ (391.40): C, 61.37; H, 3.35; N, 10.74%. Found: C, 61.12; H, 3.09; N, 10.54%.

10-Acetyl-9-phenyl-9,10-dihydrochromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (23). Compound **20** (0.31 g, 1 mmol) was dissolved in Ac_2O (15 mL) and heated under reflux for 8 h, and the reaction mixture was concentrated to small volume, poured onto ice cold H_2O, stirred for further 2 h, and refrigerated for an overnight. The separated product was filtered, washed with H_2O, dried and recrystallized from DMF/EtOH (1:1) to afford **23** (77%), as greenish solid, mp 175−177 ºC; IR (KBr): (cm^{-1}) 1635 (C=N$_{str.}$), 1665, 1685, 1725 (3C=O$_{str.}$); ^{1}H NMR (300 MHz, DMSO−d_6): δ 2.91 (s, 3H, COMe), 7.25–7.85 (m, 10H, 9Ar-H and Triaz.$_{(C5)}$-H); MS (*m/z*, %): 359.09 (M$^+$, 55%); Anal. Calcd for $C_{20}H_{13}N_3O_4$ (359.33): C, 66.85; H, 3.65; N, 11.69%. Found: C, 66.62; H, 3.41; N, 11.54%.

General procedure for preparation of pyrazole derivatives 25 and 26. To a solution of **20** (0.31 g, 1 mmol) in EtOH (30 mL) containing piperidine (0.5 mL), malononitrile (0.06 g, 1 mmol) and/or ethyl cyanoacetate (0.11 mL, 1 mmol) was added, the reaction mixture was refluxed for 8 h. then left to cool at rt. The solid product was filtered off, washed with MeOH and recrystallized from EtOH to afford **25, 26.**

1-(5-Amino-4-cyano-3-phenyl-2,3-dihydro-1*H*-pyrazole-1yl)chromeno[3,4-*c*]pyrrole-3,4-dione (25). Yellow crystals (81%), mp 168–170 ºC; IR (KBr): (cm^{-1}) 1620 (C=N$_{str.}$), 1665, 1715 (2C=O$_{str.}$), 2225 (C≡N$_{str.}$), 3235-3480 (N-H$_{str.}$ and N-H$_{2str.}$); ^{1}H NMR (300 MHz, DMSO–d_6): δ 4.81 (s, H, Pyraz.$_{(C5)}$-H), 6.73 (br, s, 2H, NH_2, D_2O-exchangeable), 7.35–7.82 (m, 9H, Ar-H), 11.42 (br, s, 1H, NH, D_2O-exchangeable); MS (*m/z*, %): 383.10 (M$^+$, 75%); Anal. Calcd for $C_{21}H_{13}N_5O_3$ (383.36): C, 65.79; H, 3.42; N, 18.27%. Found: C, 65.48; H, 3.21; N, 18.11%.

Ethyl 5-amino-1-(3,4-dioxo-3,4-dihydrochromeno[3,4-*c*]pyrrol-1-yl)-3-phenyl-2,3-dihydro-1*H*-pyrazole-4-carboxylate (26). Yellow crystals (75%), mp 258–260 ºC; IR (KBr): (cm^{-1}) 1620 (C=N$_{str.}$), 1665, 1685, 1715 (3C=O$_{str.}$), 3235-3480 (N-H$_{str.}$ and N-H$_{2str.}$); ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.71 (t, J = 6.02 Hz, 3H, $CH_2\underline{CH_3}$), 2.84 (q, J = 7.01 Hz, 3H, $\underline{CH_2}CH_3$), 4.92 (s, H, Pyraz.$_{(C5)}$-H), 6.73 (br, s, 2H, NH_2, D_2O-exchangeable), 7.35–7.82 (m, 9H, Ar-H), 11.42 (br, s, 1H, NH, D_2O-exchangeable); MS (*m/z*, %): 430.13 (M$^+$, 5%); Anal. Calcd for $C_{23}H_{18}N_4O_5$ (430.41): C, 64.18; H, 4.22; N, 13.02%. Found: C, 64.01; H, 4.12; N, 12.87%.

9-(Thiourea-1-yl)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (27). To a solution of **4g** (0.25 g, 1 mmol) in 20 mL AcOH, ammonium thiocyanate (0.08 g, 1 mmol) was added and the reaction mixture was refluxed for 10 h. The reaction mixture was cooled to rt, then poured onto ice–cooled H_2O. The precipitate was filtered off, washed with H_2O (100 mL), dried and recrystallized from MeOH to afford **27** (75%) as yellow brown crystals, mp 182–184 ºC; IR (KBr): (cm^{-1}) 1325 (C=S$_{str.}$), 1610-1620 (2C=N$_{str.}$), 1665, 1726 (2C=O$_{str.}$), 3321-3436 (N-H$_{str.}$ and NH$_{2str.}$); ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.35–7.82 (m, 4H, Ar-H), 6.94 (br, s, 2H, NH_2, D_2O-exchangeable), 12.14 (br, s, H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO–d_6): 117.4, 120.2, 125.5, 126.9, 128.3, 129.4, 154.3, 172.7 (8C=C), 143.6, 154.3 (2C=N), 159.4, 190.2 (2C=O), 181.5 (C=S); MS (*m/z*, %): 313.03 (M$^+$, 20%); Anal. Calcd for $C_{13}H_7N_5O_3S$ (313.29): C, 49.84; H, 2.25; N, 22.35%. Found: C, 49.62; H, 2.10; N, 22.14%.

9-((4-Oxo-4,5-dihydrothiazol-2-yl)amino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (28). To mixture of **27** (0.62 g, 2 mmol) and chloroacetic acid (0.18 g, 2 mmol) in AcOH (25 mL), anhydrous AcONa (0.5 g) was added. The reaction mixture was heated on water bath for 3 h, and then poured into ice-cold water. The resulting precipitate was filtered off, dried and purified by recrystallization from EtOH to afford **28** (80%) as yellow crystals, mp 232–234 ºC; IR (KBr): (cm^{-1}) 1610-1620 (3C=N$_{str.}$), 1665, 1704, 1715 (3C=O$_{str.}$), 3242 (N-H$_{str.}$); ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.91 (s, 2H, Thiazol.$_{(C5)}$-H_2), 7.35–7.82 (m, 4H, Ar-H), 11.14 (br, s, H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO–d_6): 36.5 (CH_2), 117.4, 120.2, 125.5, 126.9, 128.3, 129.4, 154.3, 172.7 (8C=C), 143.6, 154.3, 158.3 (3C=N), 159.4, 176.0, 190.2 (3C=O); MS (*m/z*, %): 353.02 (M^+, 5%); Anal. Calcd for $C_{15}H_7N_5O_4S$ (353.31): C, 50.99; H, 2.00; N, 19.82%. Found: C, 50.78; H, 1.890; N, 19.52%.

9-((5,5-Dibromo-4-oxo-4,5-dihydrothiazol-2-yl)amino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (29). To a solution of **28** (0.31 g, 1 mmol) and AcONa (0.24 g, 3 mmol) in AcOH (20 mL), bromine (0.79 mL, 10 mmol) was added dropwise. The solution was allowed to stir for 2 h at rt. Upon completion of the reaction (TLC), the reaction mixture was diluted with H_2O (100 mL). The solid was filtered off, washed with H_2O, dried and recrystallized from EtOH to yield **29** (81%) as brown solid, mp 225–227 ºC; IR (KBr): (cm^{-1}) 1610-1620 (3C=$N_{str.}$), 1665, 1704, 1715 (3C=$O_{str.}$), 3242 (N-$H_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 3.91 (s, 2H, Thiazol.$_{(C5)}$-H_2), 7.35–7.82 (m, 4H, Ar-H), 11.16 (br, s, H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO–d_6): 71.1 (C-5-Thiaz.), 117.4, 120.2, 125.5, 126.9, 128.3, 129.4, 154.3, 172.7 (8C=C),143.6, 154.3, 158.5 (3C=N), 159.4, 176.1, 190.2 (3C=O); MS (*m/z*, %): 515.84 (M^++4, 10), 513.84 (M^++2, 27), 511.84 (M^+, 25); Anal. Calcd for $C_{15}H_5Br_2N_5O_4S$ (511.10): C, 35.25; H, 0.99; Br, 31.27; N, 13.70%. Found: C, 35.01; H, 0.08; Br, 31.11; N, 13.43%.

9-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (30). A mixture of **28** (0.31 g, 1 mmol) and benzaldehyde (0.10 mL, 1 mmol) in EtOH (25 mL) containing 0.1 mL of piperidine as catalyst was refluxed for 5 h. The reaction mixture was cooled to rt., then poured onto ice–cooled H_2O. The precipitate was filtered off, washed with H_2O and the crude product was recrystallized from MeOH to afford **30** (78%) as brown crystals, mp 154–156 ºC; IR: (cm^{-1}) 1610-1620 (3C=$N_{str.}$), 1665, 1704, 1715 (3C=$O_{str.}$), 3242 (N-$H_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 7.15–7.84 (m, 6H, Ar-H), 8.12 (s, 1H, =C–H), 10.94 (br, s, H, NH, D_2O-exchangeable); MS (*m/z*, %): 441.05 (M^+, 45%); Anal. Calcd for $C_{22}H_{11}N_5O_4S$ (441.42): C, 59.86; H, 2.51; N, 15.87%. Found: C, 59.67; H, 2.28; N, 15.62%.

9-((4-Phenylthiazol-2-yl)amino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (31). An equimolar mixture of **27** (0.31 g, 1 mmol) and 2-bromoacetophenone (0.19 g, 1 mmol) in 20 mL of EtOH containing 0.1 mL of piperidine as catalyst was refluxed for 8 h. The solvent was distilled off *in vacuo*. The resultant was separated off, washed with MeOH and recrystallized from EtOH to afford **31** (72%) as pale yellow crystals, mp 205–207 ºC; IR (KBr): (cm^{-1}) 1610-1620 (3C=$N_{str.}$), 1665, 1715 (2C=$O_{str.}$), 3276 (N-$H_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 6.86 (s, 1H, Thiazol.$_{(C5)}$-H),7.05–7.78 (m, 9H, Ar-H), 11.14 (br, s, H, NH, D_2O-exchangeable); MS (*m/z*, %): 413.06 (M^+, 20%); Anal. Calcd for $C_{21}H_{11}N_5O_3S$ (413.41): C, 61.01; H, 2.68; N, 16.94%. Found: C, 95.78; H, 2.42; N, 16.7%.

9-((5-Acetyl-4-methylthiazol-2-yl)amino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (32). To a solution of thioureido **27** (0.31 g, 1 mmol) in acetone (30 mL), 3-chloro-2,4-pentanedione (0.13 mL, 1 mmol) was added, and the mixture was refluxed for 6 h. Then the reaction mixture was cooled down, diluted with H_2O (50 mL), and sodium acetate (0.49 g, 6 mmol) was added, and the mixture was stirred for 15 min at rt. The precipitate was filtered off, washed with H_2O and dried, recrystallized from EtOH to afford **32** (75%) as reddish brown crystals, mp 169-171 °C; IR (KBr): (cm^{-1}) 1610-1620 ($3C{=}N_{str.}$), 1665, 1682, 1715 ($3C{=}O_{str.}$), 3276 ($N\text{-}H_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 2.51 (s, 3H, Me), 2.62 (s, 3H, COMe), 7.25–7.81 (m, 4H, Ar-H), 10.95 (br, s, H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, DMSO–d_6): 16.6 (Me), 26.7 (COMe), 117.4, 120.2, 125.5, 126.9, 128.3, 129.4, 132.4, 154.3, 156.4, 172.7 (10C=C), 143.6, 154.3, 159.3 (3C=N), 159.4, 190.2, 197.0 (3C=O); MS (*m/z*, %): 393.05 (M^+, 15%); Anal. Calcd for $C_{18}H_{11}N_5O_4S$ (393.38): C, 54.96; H, 2.82; N, 17.80%. Found: C, 54.68; H, 2.53; N, 17.46%.

9-((5-(3-(Dimethylamino)acryloyl)-4-methylthiazol-2-yl)amino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (33). A mixture of **32** (0.39 g, 1 mmol) and DMF–DMA (0.11 mL, 1 mmol) in dry xylene (30 mL) was refluxed for 5 h. The solvent was distilled off *in vacuo* and the residual brown viscous liquid was taken in Et_2O. The resulting yellowish brown solid was filtered, washed thoroughly with Et_2O, dried then recrystallized from EtOH to afford **33** (70%) as yellowish brown powder, mp 237–239 °C; IR (KBr): (cm^{-1}) 1620-1625 ($3C{=}N_{str.}$), 1665, 1715, 1721 ($3C{=}O_{str.}$), 3219 ($N–H_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 3.21 (s, 3H, Me), 3.22 (s, 3H, Me), 3.22 (s, 3H, Me), 5.61 (d, J = 7.01 Hz, 1H, =C–H), 7.25–7.81 (m, 4H, Ar-H), 8.72 (d, J = 7.01 Hz, 1H, =CH-N), 10.94 (br, s, H, NH, D_2O-exchangeable); MS (*m/z*, %): 448.10 (M^+, 25%); Anal. Calcd for $C_{21}H_{16}N_6O_4S$ (448.45): C, 56.24; H, 3.60; N, 18.74%. Found: C, 56.11; H, 3.35; N, 18.49%.

9-((5-(5-Hydroxybenzofuran-3-carbonyl)-4-methylthiazol-2-yl)amino)chromeno[4',3':3,4]pyrrolo[2,1-*c*][1,2,4]triazole-6,7-dione (37). To a stirred solution of the enaminone **33** (0.44 g, 1 mmol) in AcOH (50 mL), 1,4-benzoquinone (0.10 g, 1 mmol) was added and the reaction mixture was stirred overnight at rt. The reaction mixture was evaporated in *vacuo*, and the solid product obtained was filtered off and recrystallized from EtOH to afford **37** (82%) as reddish brown crystals, mp 287–289 °C; IR (KBr): (cm^{-1}) 1620-1625 ($3C{=}N_{str.}$), 1665, 1672, 1715 ($3C{=}O_{str.}$), 3202 ($N–H_{str.}$), 3432 ($O–H_{str.}$); ^1H NMR (300 MHz, DMSO–d_6): δ 2.47 (s, 3H, Me), 7.25–7.81 (m, 7H, Ar-H), 8.71 (s, 1H, furan), 9.62 (s, 1H, O-H, D_2O-exchangeable), 11.25 (br, s, H, NH, D_2O-exchangeable); MS (*m/z*, %): 511.06 (M^+, 35%); Anal. Calcd for $C_{25}H_{13}N_5O_6S$ (511.47): C, 58.71; H, 2.56; N, 13.69%. Found: C, 58.41; H, 2.25; N, 13.32%.

CYTOTOXICITY ASSESSMENT

METHODOLOGY

CELL CULTURE

Two mammalian cell lines HeLa and MCF-7, a human cervical and human breast carcinoma cell line were 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% CO_2. The cells were maintained as “monolayer culture” by serial sub-culturing.

SRB CYTOTOXICITY ASSAY

Preliminary cytotoxicity was performed using SRB method as previously described.[45,46] 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 (1000, 500, 200, 100, 50, 20, 10 μg/mL). Following 72 h treatment, the cells will be fixed with 10% trichloroacetic acid for 1 h at 4 ºC. Wells were stained for 10 min at room temperature with 0.4% with Sulfo-Rhodamine-B stain (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 color intensity of each well was measured spectrophotometric-ally at 564 nm with an ELISA micro-plate reader (ChroMate-4300, FL, USA). The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line. The dose response curve of compounds was analyzed using E max model. IC_{50} was defined as the drug concentration required to decrease fluorescence to 50% of that of the control.

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