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Utilization of cyanoacetohydrazide and 2-(1,3-dioxoisoindolin-2-yl) acetyl chloride in the synthesis of some novel anti-proliferative heterocyclic compounds

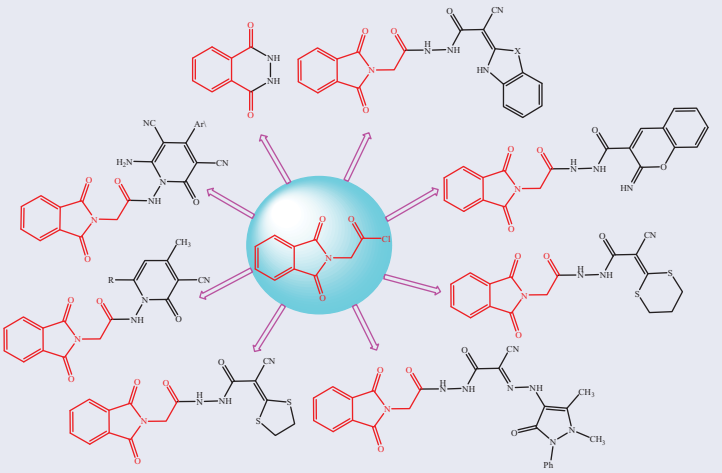
Mohamed H. Hekal, Yasmeen M. Ali, and Fatma S. M. Abu El-Azm

Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

ABSTRACT

Owing to its high reactivity and commercial availability, 2-cyanoacetohydrazide can be utilized as a versatile and appropriate intermediate for synthesis of a broad variety of heterocyclic compounds. Thus, 2-cyanoacetohydrazide and 2-(1,3-dioxoisoindolin-2-yl) acetyl chloride were used as starting materials for construction of new heterocyclic compounds bearing 1,3-dioxoisoindoline moiety. The newly synthesized compounds were recognized by elemental analyses and spectral data (IR, ^{1}H-NMR, and ^{13}C-NMR spectra). The synthesized compounds were screened for their anti-proliferative activity against two human epithelial cell lines; breast (MCF-7) and liver (HepG2) as well as to normal fibroblasts (WI-38). The data showed distinctly that compounds **1** and **12** presented promising *in-vitro* anti-proliferative activity against two cell lines (MCF-7 and HepG2) without harming normal fibroblasts.

GRAPHICAL ABSTRACT



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CONTACT Mohamed H. Hekal mohahekal2007@yahoo.com Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

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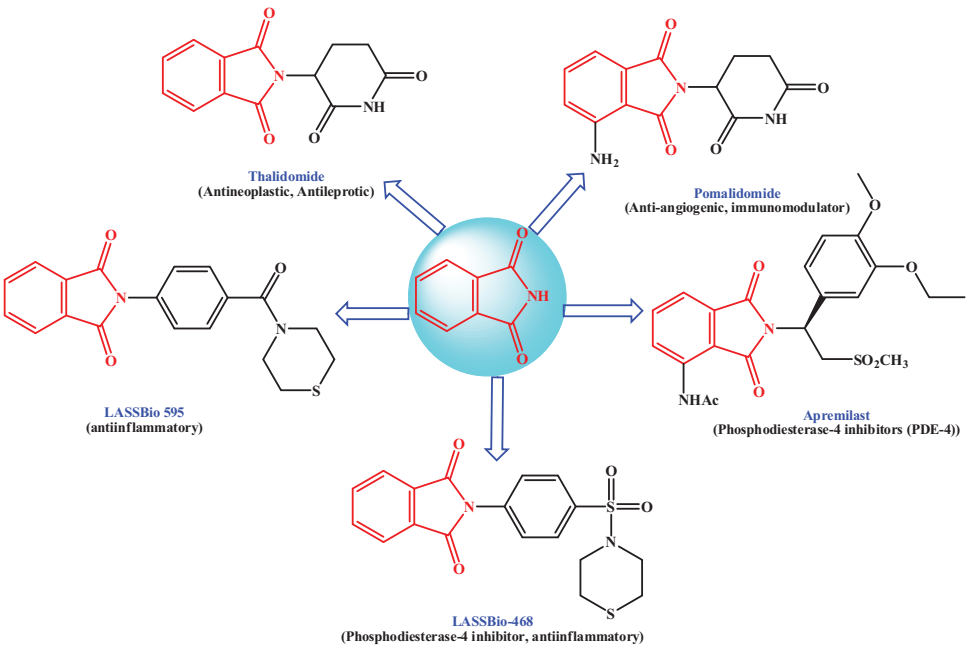


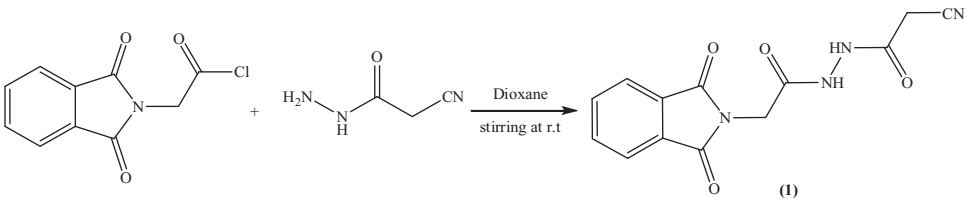
Figure 1. Several drugs bearing isoindoline-1,3-dione as a core moiety.

Introduction

Isoindoline-1,3-dione derivatives (phthalimides) act as a leading substructure in organic synthesis for preparing various biologically active molecules.[1–4] The most important pharmacological effects that have been reported for phthalimide derivatives are anti-cancer,[5] antimicrobial, anti-oxidant,[6] anti-inflammatory,[7] glucosidase inhibition,[8] antimalarial,[9] anti-angiogenic,[10] antitubercular activities,[11–13] anticonvulsant[14] and analgesic activities.[15] The structure of isoindoline-1,3-dione (-CO-N(R)-CO-) is fundamentally hydrophobic, and this raises their potential to pass biological membranes *in vivo*.[16]

The phthalimide ring has been considered as a distinctive scaffold to design novel lead drug compounds of diverse biological activities and applied for different diseases like AIDS, leprosy, diabetes, tumor, convulsion, multiple myeloma, pain, inflammation, and bacterial infection.[17,18] The highly active reference compounds such as Thalidomide (Antineoplastic, Antileprotic), Pomalidomide (Anti-angiogenic, immunomodulator), Apremilast (Phosphodiesterase-4 inhibitors (PDE-4)), LASSBio-468 (Phosphodiesterase-4 inhibitor, Anti-inflammatory) and LASSBio 595 (Anti-inflammatory) bearing phthalimide moiety that are linked to various side chain[19,20] are shown in Figure 1.

Inspection of the reference compounds which presented in Figure 1 drove us to design and synthesize some novel compounds consisting mainly of a phthalimide subunit and improved by certain pharmacophores. Therefore, to enhance the biological activity of phthalimide derivatives, a molecular crossbreeding pathway was utilized to insert different pharmacophore subunits like iminocoumarin, pyridone, 1,3-dithiolane, 1,3-dithialane and benzo- (oxazole and imidazole).



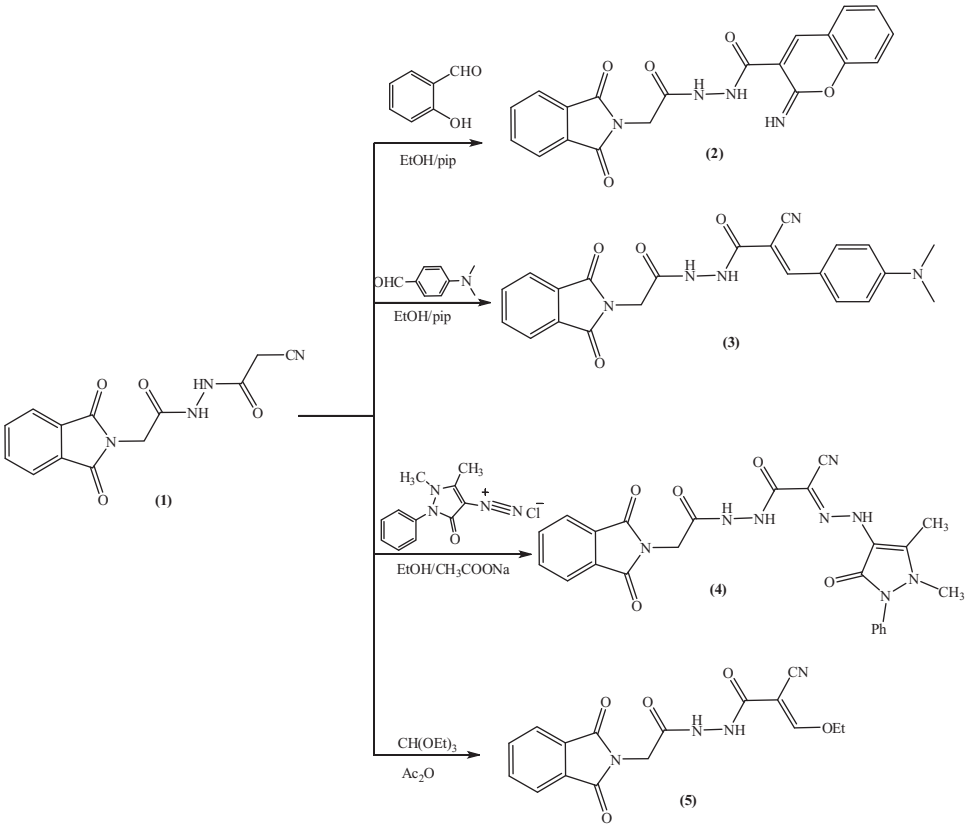
Scheme 1. Synthesis of 2-cyano-N′-(2-(1,3-dioxoisoindolin-2-yl) acetyl) acetohydrazide (1).

Among diverse commercially obtainable substituted hydrazides, 2-cyanoacetohydrazide can be considered as a versatile and appropriate intermediate for synthesis of a broad variety of heterocyclic compounds. 2-Cyanoacetohydrazide can act as an ambident nucleophile (C- and N-nucleophile). In our literary survey,[21–26] we indicate that 2-cyanoacetohydrazide has diverse functionality, which is appeared in two nucleophilic centers localized at carbon atom of active methylene group and nitrogen atom of NH_2 group, and also in two electrophilic centers localized at carbon atoms of cyano and carbonyl groups. Hence, reactions of cyanoacetohydrazide with several reactants (electrophiles and nucleophiles) are utilized in synthesis of a diversity of polyfunctional heterocyclic compounds of biological concern.[27]

Results and discussion

As an aspect of our interest in the synthesis of a broad domain of heterocyclic systems with biological applications,[28–32] our present research is interested with the exploitation of 2-cyanoacetohydrazide and 2-(1,3-dioxoisoindolin-2-yl) acetyl chloride as starting materials for construction of new heterocyclic compounds bearing 1,3-dioxoisoindoline moiety. Thus, in the current study, 2-cyano-*N*′-(2-(1,3-dioxoisoindolin-2-yl) acetyl) acetohydrazide **(1)** was obtained via stirring 2-(1,3-dioxoisoindolin-2-yl) acetyl chloride with 2-cyanoacetohydrazide in dioxane at room temperature for 30 min (Scheme 1). The chemical structure of compound **1** was concluded from the study of its spectroscopic data. Thus, the IR spectrum of compound **1** displayed υ_{NH} at 3297, 3226 cm^{-1}, $\upsilon_{C=O}$ at 1776, 1731 cm^{-1} (vibrational coupling), $\upsilon_{C=O\ (amide)}$ at 1680 cm^{-1} and $\upsilon_{C\equiv N}$ at 2263 cm^{-1}. Furthermore, the existence of two singlet signals for CH_2 protons of CH_2CN and N-CH_2CO at δ 3.72 and 4.30 ppm, respectively, together with two broad singlet signals for two NH protons at δ 10.25 and 10.32 ppm beside the aromatic protons in the ^{1}H-NMR spectrum affirm the structure **1**. Moreover, the ^{13}C-NMR spectrum of the target compound **1** exhibited signals at 23.74 ppm (CH_2CN), 66.36 ppm (N–CH_2CO), 115.56 ppm (C≡N), 123.29, 131.65, 134.68 ppm (aromatic carbons) and 161.23, 165.17, 167.37 ppm due to the presence of four CO groups which coincident with the proposed structure. The formation of compound **1** could be illustrated based on nucleophilic attack by nitrogen atom of the terminal amino group of 2-cyanoacetohydrazide on the positively polarized carbonyl carbon of the acid chloride followed by dehydrochlorination.

The functionalities in β-ketonitrile **1** made it beneficial precursor for construction of new heterocyclic compounds bearing 1,3-dioxoisoindoline moiety as the cyano and carbonyl functions are suitably located to enable reactions with prevalent reagents. Furthermore, the active methylene of β-ketonitrile **1** can participate in substitution and



Scheme 2. Condensation of the *β*-ketonitrile 1 with some electrophilic reagents.

condensation reactions. Therefore, cyclocondensation of compound **1** with salicylaldehyde in ethanolic solution containing catalytic amount of piperidine yielded the 2-imino coumarin derivative **2** (Scheme 2). The IR spectrum of compound **2** showed υ_{NH} at 3325, 3276 cm^{-1}, $\upsilon_{C=O}$ at 1775, 1727 cm^{-1} (vibrational coupling), $\upsilon_{C=O\ (amide)}$ at 1684 cm^{-1}, $\upsilon_{C=N}$ at 1640 cm^{-1} and devoid of stretching absorption band for nitrile group. Moreover, ^{1}H-NMR spectrum of the 2-imino coumarin derivative **2** showed absence of methylene protons of CH_2CN group and presence of new singlet signal assigned for C = NH proton at δ 8.45 ppm exchanged with D_2O, singlet at δ 6.24 ppm for HC_4-chromene proton beside signal for methylene protons of NCH_2 at δ 4.19, 4.25 as ABq signal with $J = 16.8$ Hz. The higher coupling constant (J) is due to their existence as geminal protons of AB system. Likewise, ^{13}C-NMR spectrum was in conformity with the suggested structure. Similarly, β-ketonitrile **1** was subjected to react with 4-(dimethylamino) benzaldehyde in ethanol containing a catalytic amount of piperidine to produce the arylidene derivative **3** (Scheme 2).

The diazonium salt of 4-aminoantipyrine was subjected to a coupling reaction with compound **1** in ethanolic sodium acetate solution at 0–5 °C to afford the corresponding hydrazinyl derivative **4** (Scheme 2). Structure of compound **4** was supported by its IR, ^{1}H-NMR and ^{13}C-NMR spectra. (cf. experimental)

2-Cyano-*N*'-(2-(1,3-dioxoisoindolin-2-yl)acetyl)-3-ethoxyacrylohydrazide **(5)** was furnished on reaction of compound **1** with triethyl orthoformate in the presence of

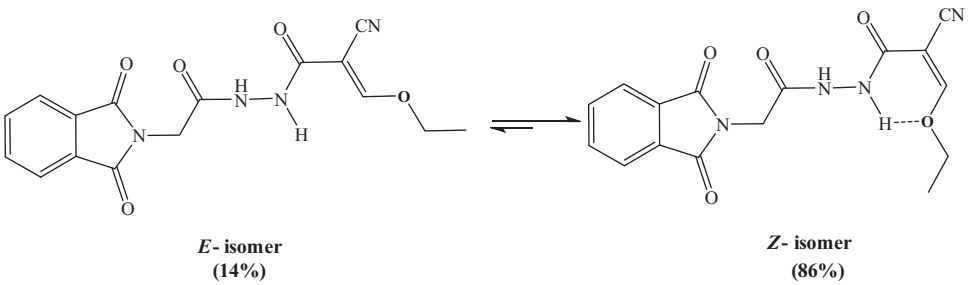


Figure 2. *E*/*Z* isomers of compound **5**.

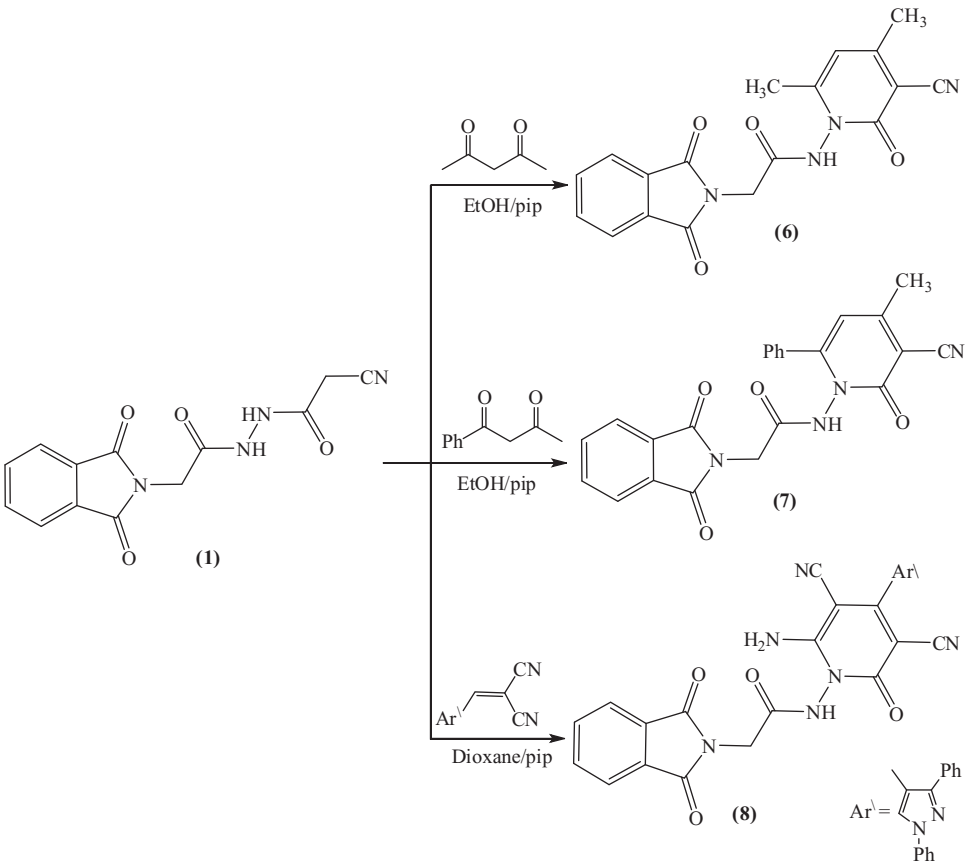
distilled acetic anhydride (Scheme 2). The suggested structure of **5** was confirmed by spectroscopic data. Compound **5** exists as *E*/*Z* mixture in the ratio 14:86 as shown from its ^{1}HNMR spectrum which exhibits duplicates of triplets and quartets in addition to two singlet signals for the olefinic proton at δ 8.22, 8.34 ppm. The higher ratio of the *Z*-isomer is due to formation of the intramolecular chelated H-bond in six-membered ring which leads to increasing its stability, as shown in Figure 2.

Moreover, ^{13}C-NMR spectrum of **5** affirmed the presence of ethoxy group carbons at δ 15.03 and 73.01 ppm.

The reactivity of acetohydrazide **1** toward β-diketones was also investigated. Therefore, subjecting compound **1** to react with acetyl acetone and/or benzoyl acetone in ethanolic solution containing catalytic amount of piperidine afforded the 2-pyridone derivatives **6** and **7**, respectively (Scheme 3). The ^{1}HNMR spectrum of compound **6** supported its structure as it displayed signals at δ 2.23 and 2.35 and 6.37 ppm attributed to protons of CH_3 and $=CH_{pyridone}$, respectively beside the absence of a singlet signal for protons of CH_2CN group. Moreover, the ^{13}C-NMR spectrum confirmed the existence of two methyl groups at δ 18.58, 20.83 ppm.

Refluxing of compound **1** with 2-((1,3-diphenyl-1*H*-pyrazol-4-yl) methylene) malononitrile in dioxane containing a catalytic amount of piperidine gave 6-amino-2-oxopyridine derivative **8** (Scheme 3). The structure of 2-pyridone derivative **8** was unambiguously achieved from analytical and spectroscopic data. For example, the IR spectrum of **8** exhibited bands for $\upsilon_{NH2,\ NH}$ at 3340, 3211, 3200 cm^{-1}, $\upsilon_{C=O}$ at 1773, 1717 cm^{-1} and $\upsilon_{C\equiv N}$ at 2215 cm^{-1}. Moreover, ^{1}H-NMR revealed the following signals at δ (ppm): 4.98 (s, 2H, NCH_2), 7.71–7.97 (m, 14H, ArH + 2H, NH_2), 8.92 (s, 1H, ArH), 11.25 (br s, 1H, NH, exchangeable with D_2O).

Compounds with active methylene moiety form carbanions in the presence of a base to react with one carbon donor such as carbon disulfide to afford dithiocarboxylates which can be transformed to ketene dithioacetals through its reaction with excess of the alkylating reagent. Consequently, stirring of the cyanoacetyl acetohydrazide **1** with carbon disulfide in the presence of potassium hydroxide in dimethylformamide yielded the nonisolable intermediate dipotassium dithiolate salt **G** which *in situ* underwent cyclodehydrobromination with 1,2-dibromoethane and/or 1,3-dibromopropane to yield dithiolane derivative **9** and dithiane derivative **10**, respectively (Scheme 4). The microanalytical and spectroscopic data were in conformity with the suggested structures of compounds **9** and **10**. For instance, ^{1}H-NMR spectrum of compound **9** detected the appearance of doublet-doublet signals at 3.58, 3.68 ppm for two SCH_2 groups with



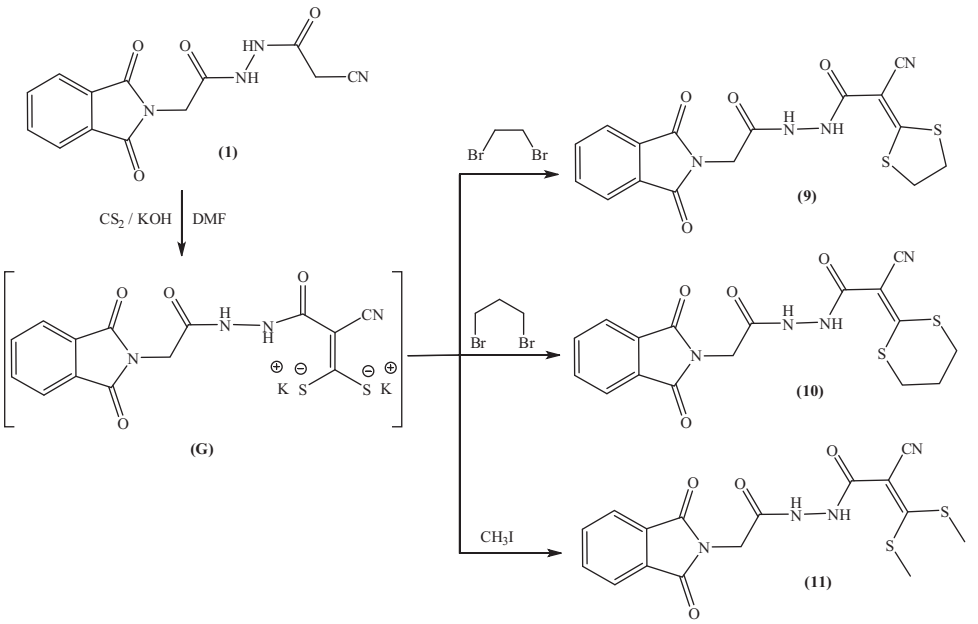
Scheme 3. Synthesis of some novel 2-pyridone derivatives from the cyanoacetohydrazide 1.

$J = 16.8$, 17.7 Hz (the protons of each methylene group are not magnetically equivalent and they undergo germinal coupling as the two CH_2 groups are in a cyclic system. This is evidenced from the higher coupling constant values). (cf. experimental)

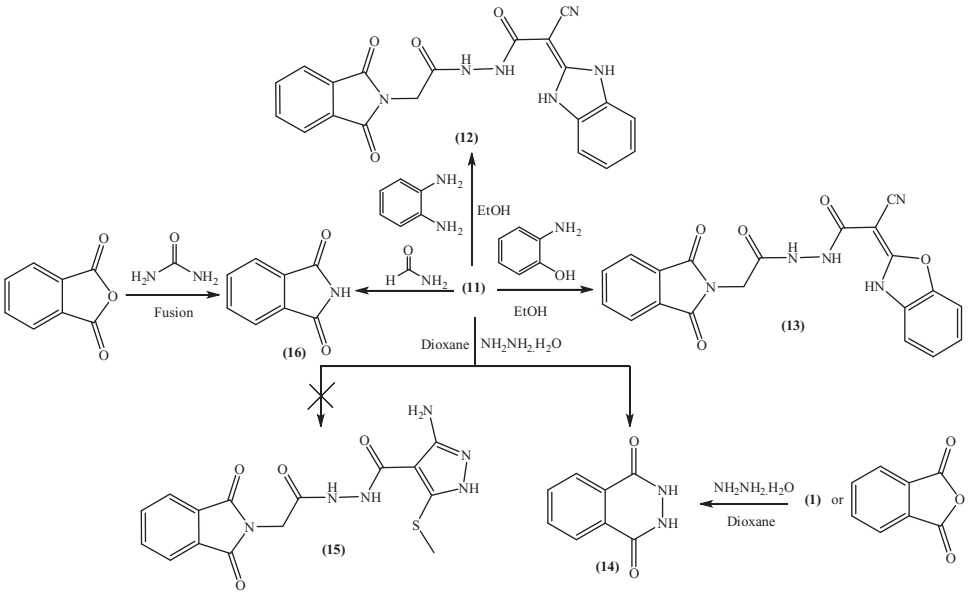
Otherwise, treatment of the dipotassium dithiolate salt **G** *in situ* with methyl iodide yielded the ketene *S,S*-dithioacetal **11** (Scheme 4). The structure of compound **11** was clarified from the elemental analysis and spectral data. Thus, the IR spectrum displayed the appearance of absorption bands at 3272, 2203 and 1776, 1721, 1694 cm^{-1} for NH, C≡N, and C = O groups, respectively. As well, the ^{1}H-NMR spectrum exhibited a singlet signal at δ 2.61 ppm for six protons of two identical *S*-methyl protons, a signal for the NCH_2 protons at δ 4.30 ppm in addition to two broad singlet signals for two NH protons at δ 10.44 and 10.52 ppm disappeared with D_2O.

Afterwards, the reactivity of the ketene *S,S*-dithioacetal **11** toward some nitrogen nucleophiles was examined. Consequently, refluxing compound **11** with bidentate nucleophiles such as *o*-phenylenediamine and/or *o*-aminophenol in absolute ethanol yielded the corresponding benzimidazole derivative **12** and benzoxazole derivative **13**, respectively (Scheme 5). Structures **12** and **13** were achieved from ^{1}H-NMR and ^{13}C-NMR spectra which revealed the absence of the two SCH_3 groups. (cf. Experimental).

Regrettably, refluxing of the ketene *S,S*-dithioacetal **11** with hydrazine hydrate in dioxane afforded sulfur-free compound which was identified as 2,3-dihydrophthalazine-



Scheme 4. Treatment of the dipotassium dithiolate salt G with some alkyl halides.



Scheme 5. Reactivity of the ketene S,S-dithioacetal 11 towards some nitrogen nucleophiles.

1,4-dione **14** rather than the respective pyrazole derivative **15**[33] (Scheme 5). The phthalazine-1,4-dione **14** was also obtained upon hydrazinolysis of the cyanoacetyl acetohydrazide **1** in refluxing dioxane. The phthalazine-1,4-dione **14** structure was proven from comparison (TLC, IR, mp) with an authentic sample prepared from reaction of phthalic anhydride with hydrazine hydrate in refluxing dioxane [39] (Scheme 5).

Table 1. Effect of the newly synthesized compounds on the viability of HepG2, MCF7 and WI-38 cells.

	IC_{50} (μM)[a]		
Compd. No.	MCF-7	HepG2	WI-38
1	2.53 ± 4.6	3.56 ± 3.6	96.44 ± 4.5
2	26.97 ± 1.9	11.26 ± 1.3	56.4 ± 4.3
3	79.91 ± 5.0	75.81 ± 4.1	72.4 ± 4.8
4	22.09 ± 1.7	21.45 ± 1.7	45.23 ± 2.9
5	64.87 ± 4.3	91.78 ± 5.0	29.5 ± 2.2
6	36.23 ± 2.8	25.89 ± 1.9	23.2 ± 1.2
7	39.48 ± 1.7	25.47 ± 4.5	49.2 ± 3.9
8	45.64 ± 0.4	34.81 ± 0.3	91.31 ± 5.1
9	58.9 ± 4.0	68.2 ± 4.4	42.2 ± 3.3
10	53.31 ± 4.0	56.04 ± 3.4	39.1 ± 2.9
11	38.4 ± 3.5	40.0 ± 3.6	44.6 ± 4.2
12	4.59 ± 0.3	5.88 ± 0.2	70.02 ± 4.6
13	17.29 ± 4.1	15.73 ± 2.8	64.29 ± 1.8
DOX	4.17 ± 0.2	4.50 ± 0.3	6.72 ± 0.5

DOX: doxorubicin.
[a]IC_{50} ((μM)): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), above 100 (non-cytotoxic).

By the same way, reaction of the ketene *S,S*-dithioac etal **11** with formamide yielded isoindoline-1,3-dione (phthalimide) **16** which was also compared with an authentic sample prepared from fusion of phthalic anhydride with urea (TLC, IR, mp)[40]. The structure of the obtained isoindoline-1,3-dione **16** was elucidated using analytical and spectroscopic data (IR, ^{1}H-NMR, and ^{13}C-NMR) (Scheme 5).

In vitro anti-proliferative activity

The *in vitro* anti-proliferative activity of the novel synthesized compounds was evaluated against breast (MCF7) and human liver (HepG2) cancer cell lines as well as to normal fibroblasts (WI-38) and compared to the activity of doxorubicin as a standard drug (Table 1; Figure 3). The report showed that doxorubicin had an IC_{50} of ∼4–7 μM against all cells investigated with no discrimination between cancer and normal cells. The novel cyanoacetyl acetohydrazide derivative **1** displayed promising anti-proliferative activity against cancer cell lines with IC_{50} of ∼2.5–3.5 μM which had much higher effective anti-proliferative activity than the activity of doxorubicin. Likewise, the novel benzimidazole derivative **12** showed almost equal activity to doxorubicin against cancer cell lines with IC_{50} of ∼4.5–5.8 μM. Compounds **1** and **12** were safe to the normal fibroblasts with IC_{50} at ∼70–96 μM. Compounds **1** and **12** showed very strong activity, this is due to the presence of two NH groups in **1** and four NH groups in **12** which may be added to any unsaturated moiety in DNA or forming hydrogen bonds with either one of the nucleobases of the DNA and causes it damage. On the other hand, compound **2** exhibited strong cytotoxic activity against HepG2 cell line with IC_{50} (11.26 ± 1.3) μM, while compound **13** exhibited strong cytotoxic activity against MCF7 cell line with IC_{50} (17.29 ± 4.1) μM. Moderate activity toward HepG-2 and MCF-7 cell lines was observed with compounds **4**, **6–8** and **11**.

2′-C-Cyano-2′-deoxy-1-β-D-arabinopentofuranosylcytosine (CNDAC)[34] is a nucleoside analogue with a novel mechanism of action that is being assessed in

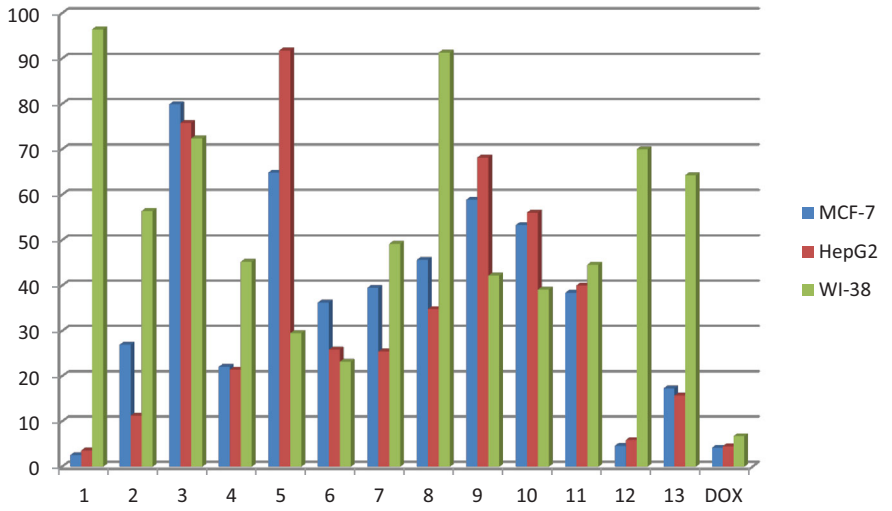
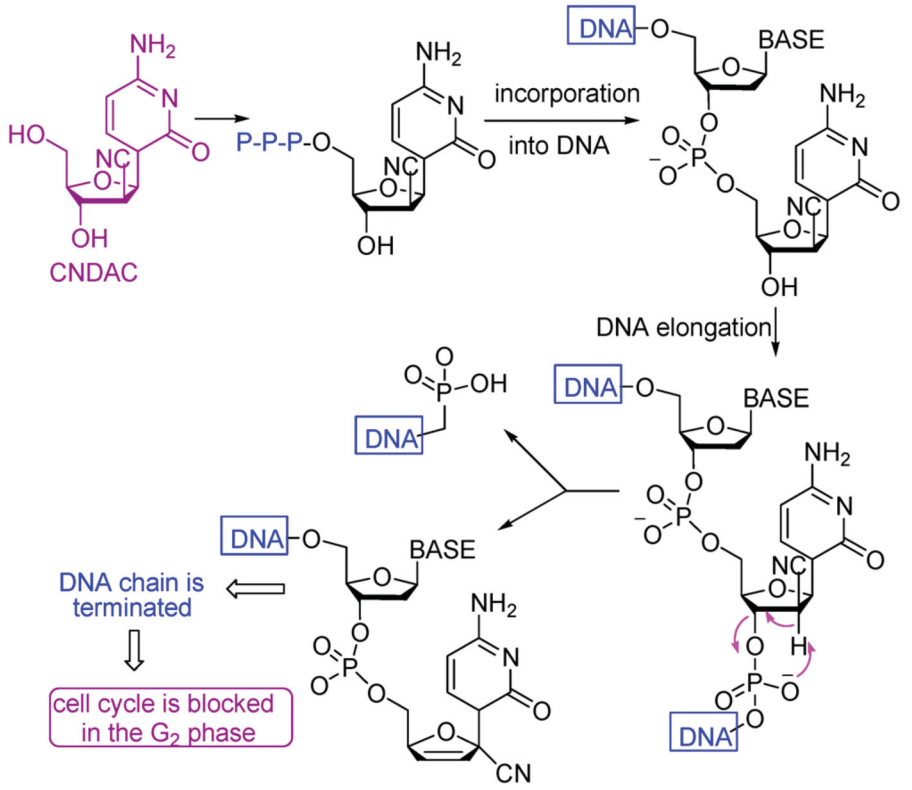
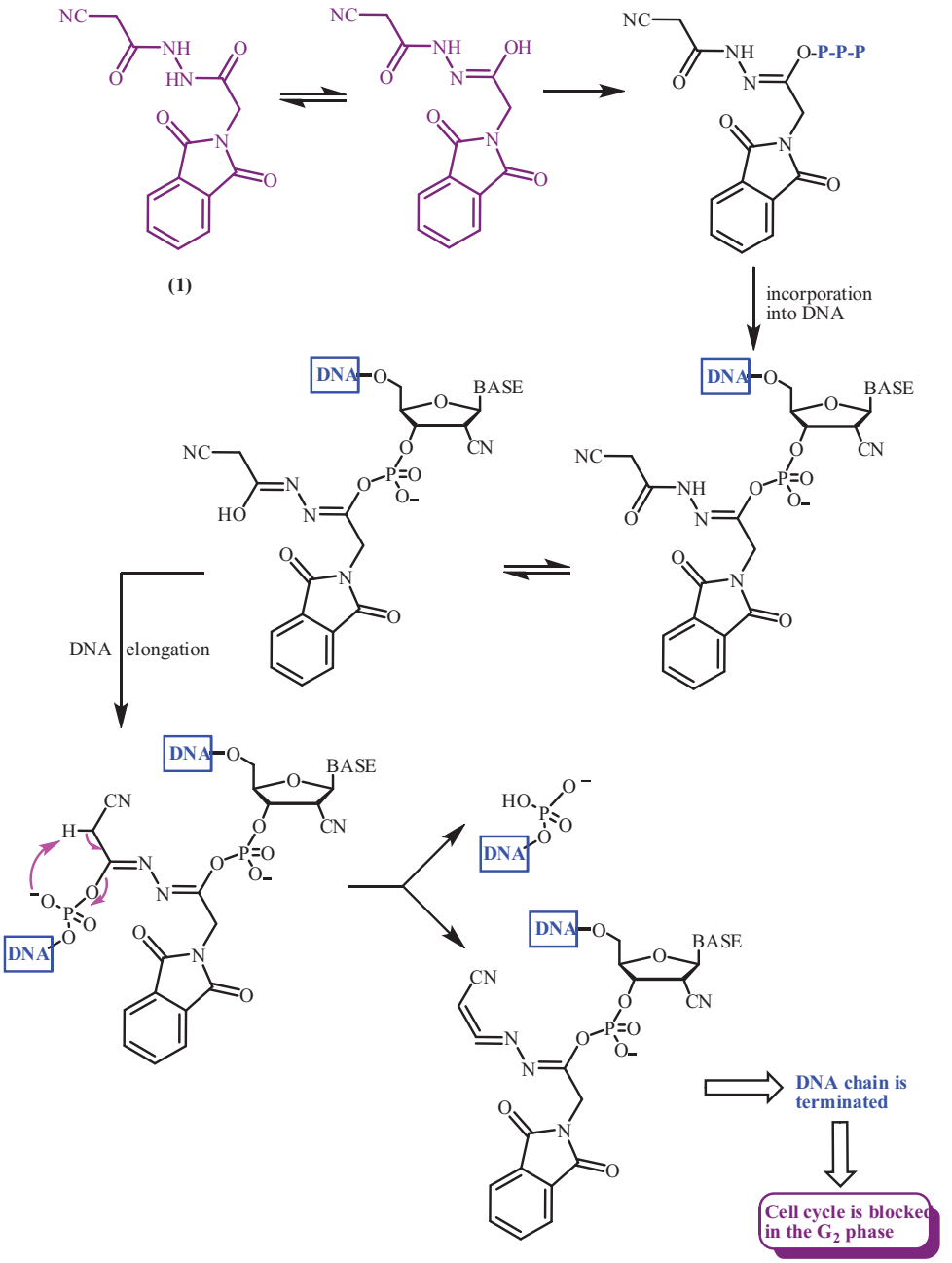


Figure 3. Cytotoxic activity of the tested compounds on different cell lines.



Scheme 6. Mechanism of the antitumor action of CNDAC.



Scheme 7. Mechanism of the antitumor action of compound **1**.

clinical experiments. Incorporation of CNDAC triphosphate into DNA and extension during replication leads to single-strand breaks directly caused by β-elimination. These breaks, or the lesions that emerge from more processing, cause cells to arrest in G_2. The electron withdrawing effect[35] of the cyano group at the arabinose 2′-β-position rises the acidity of the 2′-α proton and facilitates a β-elimination reaction involving an oxygen of the phosphate group at the 3′-β position that leads

to single strand break that affords a DNA molecule lacking a 3′-hydroxyl, which blocks its repair by ligation and leads to suppression of the cell cycle at the G_2 phase (Scheme 6).

The suggested mechanism of DNA interaction with compound **1** may be similar to the mechanism of DNA interaction with CNDAC and it is represented in Scheme 7.

Experimental

All melting points were measured on a Griffin and Georgy melting-point apparatus (Griffin & Georgy Ltd., Wembley, Middlesex, UK) and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer (Pye Unicam Ltd., Cambridge, UK) by using the KBr wafer technique. ^{1}H-NMR spectra were determined on a Varian Gemini 300 MHz on Bruker Avance III using tetramethylsilane as an internal standard (chemical shifts in δ scale), while ^{13}C NMR spectra were run at 100 MHz. Elemental analyses were carried out at the Microanalytical Unit, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer (Waltham, MA), and satisfactory analytical data (±0.4) were obtained for all compounds. The homogeneity of the synthesized compounds was controlled by thin layer chromatography (TLC), using aluminum sheet silica gel F_{254} (Merck). The antitumor activities were performed at Micro analytical Center of Mansoura University, Egypt.

2-Cyano-N'-(2-(1,3-dioxoisoindolin-2-yl) acetyl) acetohydrazide (1). A mixture of 2-(1,3-dioxoisoindolin-2-yl) acetyl chloride (2.2 g, 10 mmol) and 2-cyanoacetohydrazide (0.99 g, 10 mmol) was stirred in dioxane (20 ml) at room temperature for 30 min. The deposited solid on stirring was filtered off, dried and recrystallized from EtOH/dioxane mixture to give **1** as white crystals; mp: 258–260°C, yield: 80%. IR (KBr, ν, cm^{-1}): 3297, 3226 (NH), 3058 (CH aromatic), 2977, 2923 (CH_2 aliphatic), 2263 (C≡N), 1776, 1731, 1680 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.72 (s, 2H, CH_2CN), 4.30 (s, 2H, NCH_2), 7.85-7.93 (m, 4H, ArH), 10.25 (br s, 1H, NH, exchangeable with D_2O), 10.32 (br s, 1H, NH, exchangeable with D_2O). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 23.74, 66.36, 115.56, 123.29, 131.65, 134.68, 161.23, 165.17, 167.37. Anal. Calc. for $C_{13}H_{10}N_4O_4$ (286.24): C, 54.55; H, 3.52; N, 19.57. Found: C, 54.23; H, 3.44; N, 19.50.

Pharmacological activity

Cytotoxicity assay

The cytotoxic activity of sixteen compounds was tested against two human tumor cell lines namely: mammary gland (breast) MCF-7 and hepatocellular carcinoma (liver) HePG-2 in addition to normal fibroblasts (WI-38). The cell lines were obtained from the ATCC via the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Doxorubicin was used as a standard anticancer drug for comparison. The reagents used were RPMI-1640 medium, MTT, DMSO and Doxorubicin (Sigma Co., St. Louis, MO, USA), and Fetal Bovine Serum (GIBCO, Paisley, UK). The different cell lines[36,37] mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion

of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100 µg/mL streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded[38] in a 96-well plate at a density of 1.0×10^4 cells/well at 37 °C for 48 h under 5% CO_2 incubator. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µL of MTT solution at 5 mg/mL was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µL was added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, BioTech, Winoosky, VT).

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