*Said MM et al. / Vol 6 / Issue 1 / 2016 / 35-42.*

International Journal of

Innovative Drug Discovery

e ISSN 2249 - 7609
Print ISSN 2249 - 7617

www.ijidd.com

# NOVEL N-SUBSTITUTED -1H-ISOINDOLE-1,3(2H)DIONES OF POSSIBLE GENOTOXIC AND ANTICANCER ACTIVITIES

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**ABSTRACT**

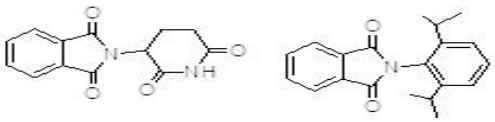
Several N-(heterocyclic)-1H-isoindole, were prepared from hydrazine carboxylic acid-1,3-dioxo-1,3-dihydro-isoindole-2-yl ester for the purpose of possible genotoxic activity against cancer cell line of mouse spleen. In conclusion, the correlation between chromosomal aberrations induction, suppression of proliferation rate and antitumor activity observed in this study, appeared to further substantiate the validity of the chromosomal aberration assay as a possible method for improving and guiding chemotherapy.

**KEY WORDS**: Isoindol-1,3-diones, hydrazides ,Schiff bases, Thiadizines, Mannich bases ,Genotoxicity, Spleen cancer cell line, Anticancer evaluatiuon, cyclophosphamide.

## INTRODUCTION

Several phthalimide derivatives have been reported to posses improved activities against L-1210 lymphoid leukemia, colorectal adenocarcinoma SW480, lung bronchogenic MB-981, Hela-$S_3$ suspended cervical carcinoma and glioma EH118, MG cell lines with $ED_{50}$ at concentrations ranged between 1.16-2.57µg/ml [1-4].

In 1998, Thalidomide;2-(2,6)-dioxopiperidin-3yl)-1H-isoindole-1,3-(2H)dione I [5] was approved by the United State Food and drug Administration (FDA) for the treatment of erythua nodosum leprosum (ENL) and On 2006, it granted accelerated approval for using this drug in combination with dexamethazone for the treatment of newly diagnosed multiple myeloma (MM) patients. Other researches has shown promising results with thalidomide in patients with myelodysplastic syndrome, a variety of infectious diseases, autoimmune diseases, prostate cancer, Kaposi's sarcoma and multiple myeloma, progressive body weight loss related to advanced cancer and Aids, antiangiogenesis and significantly inhibits metastasis [6-11]. Several thalidomide analogs were synthesized and tested as antiangiogenic agents for example: [2-(2,6-di(propan-2-yl)phenyl]-5-hydroxy-1H-isoindole-1,3(2H)-dione] **II** showed the most potent activity against angiogenesis which is a primary target of anticancer therapy nowadays [9]. Angiogenesis refers to process of generating new capillary blood vessels from pre-existing blood vessels. This process is required for a variety of physiologic processes, including normal growth and development, reproduction and wound heals. Inhibition of angiogenesis is needed to treat patients with cancer, based on the demonstration that tumor growth is angiogenesis dependent. Blocking tumor induced angiogenesis contributes to lay an attractive strategy in cancer therapy.




I II

In this work several isoindolone derivatives were synthesized for their genotoxicity and anticancer evaluation on cultured spleen cancer cells from mice.

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Antineoplastic drugs are responsible for the survival of cancer patients around the world. However, like many other cancer therapeutics, they themselves may cause paclitaxel) as the most important new cancer drug in the mutations and secondary malignancies [12]. Cancer induction is therefore a toxic consequence predicted by short-term tests of genotoxicity and should be weighed against the potential therapeutic benefits of several antitumor drugs [13]. It is therefore essential that effective anticancer drugs should be tested not only for their cytotoxic potential, but also for their ability to disturb genomic integrity, in order to render a deeper understanding of the potential risks related to their clinical use [14]. To study genotoxicity, some studies [15-19] have used a variety of genetic markers, including structural chromosomal aberrations (gapes, breaks trans-location) and numerical chromosomal aberrations (polyploidy).

**Chemistry**

Reaction of N-hydroxy phthalimide with ethyl chloroformate afforded the corresponding ester **2**, which upon reaction with 90% hydrazine hydrate gave the carbohydrazide **3 (scheme 1**). Reaction of 3 with different aromatic aldehydes according to areported method **[20]** afforded the corresponding Schiff bases **4 (scheme 2).** Cyclization of compounds **4** with thioglycolic acid in the presence of zinc chloride gave the 1,3,4-thiadiazines 5 which underwent reaction with 40% formaldehyde and morpholine to give the corresponding Mannich bases 6. Also, reactionof compound **3** with isothiocyanate , gave the 2-aminothiadiazole derivative **7**, while reaction of **3** with cloroacetic acid in presence of acetic anhydride, gave the pyrrolidind-dione derivative **8**. Further, reaction of compound **3** benzaldehyde in presence of chloro acetyl chloride, afforded the corresponding phenyl azetidine derivative **9 (scheme 2)**

***EXPERIMENTAL***

All melting points are uncorrected and were taken in open capillary tubes using an Electrothermal IA 9100 digital melting point apparatus. Elemental microanalyses were carried out at Microanalytical Laboratory, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, on Vario Elementar analyzer and were found within ± 0.5% of the theoretical values. Infrared spectra were recorded on a FT/IR-6100, Fourier transform, infrared spectrometer using KBr disc technique. 1H NMR was determined by using a JEOL AS-500 NMR spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, chemical shifts are expressed in δ (ppm) downfield from TMS as an internal standard. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected using UV lamp at λ254 nm. The chemical names given for the prepared compounds are according to the IUPAC system. The biological evaluation of some of the new compounds was screened at Functional genome analysis, German cancer research center (DKFZ), INF 580, 69120 Heidelberg, Germany.

***Hydrazine Carboxylic acid 1,3-dioxo-1,3-dihydro-isoindol-2-yl ester ( 3 )*:**

N-hydroxyphthalimide (1.6 g, 0.01 mol ) and ethyl chloro formate ( 0.011 mol ) were refluxed for 4hrs. in acetone in the presence of potassium carbonate.The resulted precipitate. was refluxed with hydrazine hydrate ( 90% ) in the presence of absolute ethanol; the ppt was filtered off; crystallized from ethanol; yield 75%; m.p 212ºC. IR spectra showed bands at 1680 cm-$^{1}$ (C=O); m.s = 221. NMR spectra showed signals at δ 2.0 (s, 2H, NH2) and at δ 7.8-8 (s, 1H, NH and m, 4H ar.). Elemental analysis Calc/Found: C:48.87/48.00; H: 3.19/3.00; N; 19.00/18.78.

***N'-(4-Nitro-benzylidene)-hydrazinecarboxylic acid 1,3-dioxo-isoindol-2-yl ester (4a); N'-Thiophen-3-ylmethylene-hydrazinecarboxylic acid 1,3-dioxo-isoindol-2-yl ester (4b); N'-(2-Hydroxy-benzylidene)-hydrazinecarboxylic acid 1,3-dioxo-isoindol-2-yl ester (4c); N'-(4-Hydroxy-benzylidene)-hydrazinecarboxylic acid 1,3-dioxo-isoindol-2-yl ester (4d)*:**

These compounds were prepared as the reported method [20] by heating ethanolic solution of equimolecular amounts of compound 3 and the appropriate aromatic aldehyde: 4-nitrobenzldehyde, thiophene-3-carboxaldehyde, 2-hydroxy- and/or 4-hydroxybenzaldehyde. Compound **4a** :Yield 65%, mp 227 $^{0}$C, **IR** ($\nu$ cm$^{-1}$): 3168(NH), 2898 (aromatic-CH) and 1658 (CO); **MS**: 354.44 ($M^{+}$, corresponding to the molecular weight of the molecular formula $C_{16}H_{10}N_4O_6$ of the assigned structure), Anal.for $C_{16}H_{10}N_4O_6$ : Calcd./Found (%): C(57.38/57.45%), H(2.82/3.22%), N(15.80/15.43%). Compound **4b** : Yield 75%, mp 293 $^{0}$C, **IR** ($\nu$ cm$^{-1}$): 2895 (NH), 3057 (aromatic-CH) and 1661 (CO); **MS**: 335($M^{+}$,3%) Anal. for $C_{14}H_9N_3O_4S$ : Calcd./Found (%): C(55.62/55.42%),H(2.81/3.26%),N(12.61/12.35%).1HNMR :δ4.0($NH_2$);δ78.1(ar.).Compound **4c**: Yield 65%, mp 227 $^{0}$C, **IR** ($\nu$ cm$^{-1}$): 3168(NH), 3057 (aromatic-CH) and 1658 (CO); **MS**: 325.44 ($M^{+}$, 3%) ;Anal. for $C_{16}H_{11}N_3O_5$ : Calcd./Found (%): C(59.07/58.78%), H(3.38/4/3.77%), N(12.90/12.87%). Compound **4d** : Yield 75%, mp 220$^{0}$C, **IR** ($\nu$ cm$^{-1}$): 3168(NH), 3057 (aromatic-CH) and 1661 (CO); **MS**: 325 ($M^{+}$, 4%), Anal. for $C_{16}H_{11}N_3O_5$ : Calcd./Found (%): C(59.09/59.18%), H(3.38/3.10%), N(12.91/12.36%). $^{1}$H-NMR : δ2.51(s,3H,$CH_3$); δ4.3 (s,1H,CH); δ7.1-8.1(m,8H,ar).

***1,3-dioxoisoindolin-2-yl 2--(4-nitrophenyl)- -5-oxo-5,6-dihydro-4H-1,3,4-thiadiazine-4-carboxylate(5a);1,3-dioxoisoindolin-2-yl-5-oxo-2-(thiophen-3-yl))-5,6-dihydro-4H-1,3,4-thiadiazine-4-carboxylate-(5b);1,3-***

***dioxoisoindolin-2-yl-2-(2-hydroxyphenyl))-5-oxo-5,6-dihydro-4H-1,3,4-thiadiazine-4-carboxylate(5c);1,3-dioxoisoindolin-2-yl-2-(4-hydroxy-phenyl))-5-oxo-5,6-dihydro-4H-1,3,4-thiadiazine-4-carboxylate(5d) :***

A mixture of **(4)** ( 0.01mole ) and thioglycolic acid ( 0.01 mole ) was refluxed in 20 ml of DMF in the presence of $ZnCl_2$ for about 8hrs. The reaction mixture was cooled and poured into ice-cold water. The resulting solid was filtered and recrystallized from DMF-$C_2H_5OH$ . **5a**: (m.p 310ºC; yield 55%. IR.spectra exhibited bands at 2930 $cm^{-1}$ ( $CH_2$ ); 1720 ( C=O, thiadiazine ); 1610 $cm^{-1}$ (CO) and at 1320 ( C-S ).MS,m/z 426.4(M+,4%); Anal. for $C_{18}H_{10}N_4O_7S$ : Calcd./Found (%): C(50.7./51.00%), H(2.41/2.49%), N(11.96/11.45%); S: (6.85/6.75).$^1$H-NMR : δ2.98(s,2H,$CH_2$); δ3.76: (s,2H,$CH_2$); δ7.8-8.31(m,10H,ar.) .**5b**: (m.p >310ºC; yield 60%. IR.spectra exhibited bands at 2930 $cm^{-1}$ ( $CH_2$ ); 1720 ( C=O, thiadiazine ); 1610 $cm^{-1}$ ( CO) and at 1320 ( C-S ). MS m/z 387.4(M+ 3%); Anal. for $C_{16}H_9N_3O_5S_2$ : Calcd./Found (%): C(49.61/50.25%), H: (2.32 /2.45%), N(10.79/10.55%); S(16.51/15.95).1H-NMR : δ2.83(s,2H,$CH_2$); δ 7.8-8.13(m,7H,ar.) .**5c**: (m.p 290ºC ethanol; yield 65%. IR.spectra exhibited bands at 2930 $cm^{-1}$ ( $CH_2$ ); 1720 ( C=O, thiadiazine ); 1610 $cm^{-1}$ ( CO) and at 1320 ( C-S ).MS, m/z 397.05(M+, 2%); Anal. for $C_{18}H_{11}N_3O_6S$ : Calcd./Found (%): C(54.67/54.55%), H: (2.58/2.75%), N(10.0/9.45%); S(8.00/7.55).1H-NMR : δ2.98(s,2H,$CH_2$); δ 3.76(b,1H,aromaticOH); δ 6.88-7.47(m,7H,ar.). **5d**: (m.p 255ºC ethanol; yield 70%. IR.spectra exhibited bands at 2925 $cm^{-1}$ ( $CH_2$ ); 1710 ( C=O, thiadiazine ); 1600 $cm^{-1}$ ( CO) and at 1320 ( C-S ). Anal. for $C_{18}H_{11}N_3O_6S$ : Calcd./Found (%): C(54.67/54.45%), H: (2.58/2.88%), N(10.0/9.50%); 1H-NMR : δ3.76(s,2H,$CH_2$); δ5.35 (b,1H,aromatic OH); δ 6.88-7.77(m,8H,ar.).

***2-(4-Nitro-phenyl)-5-morpholinylmethyl-4-oxo-thiadiazolidine-3-carboxylic acid 1,3-dioxo-1.3-dihydro isoindol-2ylester(6a);2--thiophen-3-yl)-5-morpholinylmethyl-4-oxo-thiadiazolidine-3-carboxylic acid 1,3-dioxo-1.3-dihydro isoindol-2-yl ester (6b); 2(3-hydroxy-phenyl)-5-morpholinylmethyl-4-oxo-thiadiazolidine-3-carboxylic acid 1,3-dioxo-1.3-dihydro isoindol-2-yl ester(6c); 2-(4-hydroxyphenyl)-5-morpholin-yl-4-oxo-thiadiaszolidine-3-carboxylic acid 1,3-dioxo-1.3-dihydro isoindol-2yl ester(6d)*:**

**General method:**

An aqueous solution of 40% formaldehyde ( 0.011 mole ) was added dropwise to a mixture of **5** and morpholine ( 0.011 mole ) contained in about 40 ml of ethanol. The temperature. kept below 10ºC and the solution was effectively stirred, the reaction mixture was then refluxed for 5 hrs.and left overnight at room temperature. After removing the volatile materials under reduced pressure, the product was isolated in the form of solid free base.

**6a** :mp 200 oC from dil ethanol, MS m/z 526.08 (M+ 3%); Anal. for $C_{23}H_{19}N_5O_8S$ : Calcd./Found (%): C(52.47/52.40%), H(3.45/3.40%), N(10.64/10.50%), S: ( 6.09/6.00 ).

**6b** :mp 177 oC, dil ethanol, MS m/z 484.51(M+, 3%) ; Anal. for $C_{21}H_{18}N_4O_6S_2$ : Calcd./Found (%): C(51.74/51.63%), H(3.51/3.47%), N(8.62/8.57%), S: ( 13.15/12.90 ). 1H-NMR : δ2.37(s,2H,$CH_2$); δ4.61: (s,1H,CH); δ7.6-8.1(ar.).

**6c**:mp 180 oC from dil ethanol, MS m/z 497.00; (M+, 4%); Anal. for $C_{23}H_{20}N_4O_7S$ : Calcd./Found (%): C(55.53/55.00%), H(3.85/3.77%), S: ( 6.45/6.40 ). 1H-NMR : δ4.61(s,1H,CH); δ5.92: (s,1H,CH); δ7.6-8.1(ar.).

For **6d**: mp 211 oC from dil ethanol, MS, m/z 497.00(M+;3%) Anal. for $C_{23}H_{20}N_4O_7S$ : Calcd./Found (%): C(55.53/55.33%), H(3.85/3.74%), N(11.05/10.77%), S: ( 6.45/6.00 ). 1H-NMR : δ4.61(s,2H,$CH_2$); δ5.92 (s,1H,CH); δ7.6-8.1(ar.).

***(2-(5-Aminothiadiazol-2-yl)-5-carboxylic acid-1,3-dioxo-1,3 dihydro- isoindoline-2-yl ester ( 7 ):***

A methanolic solution of compound 3 ( 0.01 mole ) and isothiocyanate ( 0.01 mole ) was refluxed for 5 hrs. and then cooled. The resulting solid was filtered, washed and recrystallized from ethanol m.p: 242ºC, yield 75%. IR spectra showed bands at 3280 ( $NH_2$ ), 1670 ( C=O ), 1120 (-O-C ) and 1090 ( C-S- ). M.S.m/z 290.3,(M+,3%). For $C_{11}H_6N_4O_4S$ ;$^1$H-NMR: δ 4.0 (b,2H, $NH_2$ ); δ 5.13 (s,2H, $NH_2$); δ7.08-8.1(ar.)

***2-((1,3-Dioxoisoindoline-2-yl)oxy)-N-2,5-dioxopyrrolidine-1-yl) amide( 8 ):***

A mixture of compound **3** (0.01 mole) and chloro acetic acid (0.01mole) in acetic acid (20 ml), acetic anhydride (10 ml) was refluxed for 4hrs. Then the reaction mixture was cooled, poured onto ice/cold water. The formed precipitate was filtered, dried and recrystallized from ethanol to give compound **8.** Yield (75 %); mp 230 $^0$C; **IR** υ($cm^{-1}$): 3484, 3366(-NH, -$NH_2$), 2999, 2931 ($CH_2$), 2215(CN), 1663 (C=O); **MS**: $M^+$ m/z 303.5 ( 5% ); **$^1$H NMR** (DMSO-$d_2$) ($\delta$ppm):δ4.42 (2H, s, $CH_2$) ; δ 2.73 (2H, s, $CH_2$) ; δ 2.74 ( 4H, m, $2CH_2$ ) ; δ 8.0 ( 1H, s, NH ) ; δ7.6-8.1(H, m aromatic protons),Anal, for $C_{13}H_9N_3O_6$(303) Calcd./Found (%):C(51.49/51.42%), H(2.99/2.88%), N(13.86/13.78%).

***2-((1,3-Dioxoisoindoline-2-yl)oxy)-N-(3-chloro-2-oxo-4-phenylazetidin-1-yl) amide ( 9 ):***

To a solution of ( 3 ) ( 0.01 mole and triethyl amine (few drops) in dioxane ( 20 ml), chloroacetyl chloride (0.01) was added dropwise with stirring at room temperature for 1hr.,and then refluxed for 3 hrs. The resulting solid on cooling was filtered, triturated with ether : petroleuim ether (1:1) until solidfy. The solid separated was recrystallized from ethanol (m.p:190 oC,).Yield 60%.MS, m/z 385.05;(M+,4%) Anal. for $C_{18}H_{12}N_3O_5Cl$:Calcd./Found (%): C(56.04/55.90%), H(3.14/3.00%), N: (10.89/10.70). $^1$H-NMR: δ5.0(s,1H,CH); δ5.44: (s,1H,CH); δ7.1-8.1(ar.); δ8.5(b,2H,$NH_2$).

**Biological activity:**

**i. Genotoxicity**

**Chemicals**

All reagents were obtained from Sigma, St Louis, MI, USA. Except RPMI 1640 medium and fetal calf serum (Invitrogen Corp., Carlsbad, CA, USA).

**Mice**

Male albino mice 7-week-old weighing 18- 20 g were used in the present study. The experiment was done in the National Research Center, genetics and cytology Dept., in wide cages and well aerated rooms. Food and water was provided *ad libitum.* .

**Spleen cell cultures**

Mice were sacrificed under ether anesthesia immediately after induction of spleen cancer with cyclophosphamide [20], spleen was sterilely removed. Spleen cells were isolated and cultured in RPMI 1640 medium containing fetal calf serum, concanavalin A (ConA,3 g/ml), antibiotics (Penicillin and streptomycin) and under 5% $CO_2$ atmosphere with 95% humidity at 37° C.

**Toxicity evaluation of the tested compounds**

All the tested compounds (11 samples) dissolved with DMSO, A wide range of concentrations, from 1– 0.00001 mg/ml in DMSO, for each tested compound were tested. Spleen cells (16 hrs old) were treated for 4 hrs. with the different concentrations of each compound. Then the medium was removed and the cells were left to grow in a fresh medium for another 20 hrs. Harvested cells were stained with trypan blue stain (1:1 v/v), counting using Neubauer chamber under light microscope, a mean percentage of vital cells were determined from three separate experiments for each concentration of the tested compounds.

***In Vitro* chromosomal preparation**

Cancerous spleen cultured cells as a positive control, and the same cells treated with the half of the $LD_{50}$ of the tested compounds (Most toxic tested compounds) were cultured for 20 hours. Two hours prior harvesting, Colchicine was added (14µg/kg B. Wt.) for collection of chromosomes at metaphase stage. Hypotonic treatment to the cell pellet, fixation of the cells and chromosomal preparation were done with the standard air dry technique as Yosida and Amano [21]. Three separate experiments were conducted. About 500 well spread metaphase were scored per culture. Chromosomal aberrations were defined after Sharma [22].

**Statistical evaluation**

For the statistical evaluation of the experimental data, the Student's *t*-test was used. Data are presented as mean value ± standard error of the mean (SEM).

**RESULTS**

According to the results of Toxicity assay as illustrated in table 1 showed that all the tested compounds has a $LD_{50}$ ranged between 10 mg/ml – 0.008 mg/ml. It is obviously notice that the most toxic compounds were Samples 7 > 4b =6b = 6c. So, we examined the four compounds (7, 4b, 6b, 6c) on its ability of affecting on the spleen cancer cells of mouse which induced with cyclophosphamide.

We selected the descending concentrations of the $LD_{50}$ in order to evaluate the potential recovery effect on the cancer cells. These concentrations were 1/2 and 1/10 of the $LD_{50}$ for all the tested four compounds. The percentage of chromosomal aberrations which induced with the positive control (cancer spleen cells without treatment) was 14.5% as illustrated in table 2. After treatment these cell lines with the tested compounds, it was noticed that there is statistically non-significant ($P < 0.01$) effect with the low concentration (1/10 $LD_{50}$) for each compound. On the contrary, with treatment with the high dose of each compound (1/2 $LD_{50}$), it was noticed that all these concentrations induced a significant reduction to the percentage of chromosomal aberrations in the cultured cancer spleen cells, as illustrated in fig. 1.The most common types of chromosomal aberrations induced in the cultured spleen cells were polyploidy as illustrated in figure 2 and aneuploidy as numerical aberrations, and chromosomal breaks as structural types.

**Table 1. Half of lethal dose ($LD_{50}$) of the tested compounds on cultured spleen cells of mice**

| Compounds | $LD_{50}$ |
|---|---|
| 3 | 1 ug/ml |
| 4a | 0.08 ug/ml |
| 6b | 0.01 ug/ml |
| 9 | 0.03 ug/ml |
| 4c | 0.06 ug/ml |
| 4d | 10 ug/ml |
| 5c | 1 ug/ml |
| 6d | 6 ug/ml |
| 6c | 0.01 ug/ml |
| 6a | Not dissolved in DMSO |
| 7 | 0.008 ug/ml |
| 4b | 0.01 ug/ml |

**Table 2. Percentage of chromosomal aberrations in cancer spleen from mice induced with cyclophosphamide and treated with the synthetic chemicals**

| Treatment | Treated dose | Number of observed metaphases in cells | Number of observed metaphases with chromosomal aberrations | Percentage of metaphases with chromosomal aberrations (mean ± S.E.) |
|---|---|---|---|---|
| -**Non treated cancer cell** (Positive control) | - | 296 | 43 | 14.53 ± 0.36 |
| **Cancer cells treated with:** | | | | |
| Sample No. 7 | 1/10 $LD_{50}$ | 250 | 32 | 12.8 ± 0.76 |
| Sample No. 4d | 1/10 $LD_{50}$ | 250 | 34 | 13.6 ± 0.34 |
| Sample No. 6d | 1/10 $LD_{50}$ | 250 | 34 | 13.6 ± 0.89 |
| Sample No. 6c | 1/10 $LD_{50}$ | 250 | 36 | 14.4 ± 1.34 |
| | | | | |
| Sample No. 7 | 1/2 $LD_{50}$ | 250 | 22 | 8.8 ± 0.5 * |
| Sample No. 4d | 1/2 $LD_{50}$ | 250 | 24 | 9.6 ± 0.63 * |
| Sample No. 6d | 1/2 $LD_{50}$ | 200 | 21 | 10. 5 ± 1.33 * |
| Sample No. 6c | 1/2 $LD_{50}$ | 200 | 22 | 11.0 ± 1.52 * |

*Significant comparing to the non-treated positive control, at (P< 0.01) level, t-test.

**Fig. 1. Chromosomal aberrations induced in cultured cancer spleen cells post treatment with different tested compounds (Compounds, 7, 4d, 6d and 6c)**

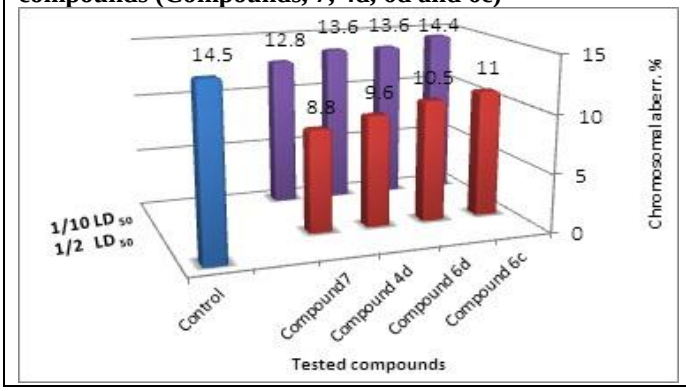
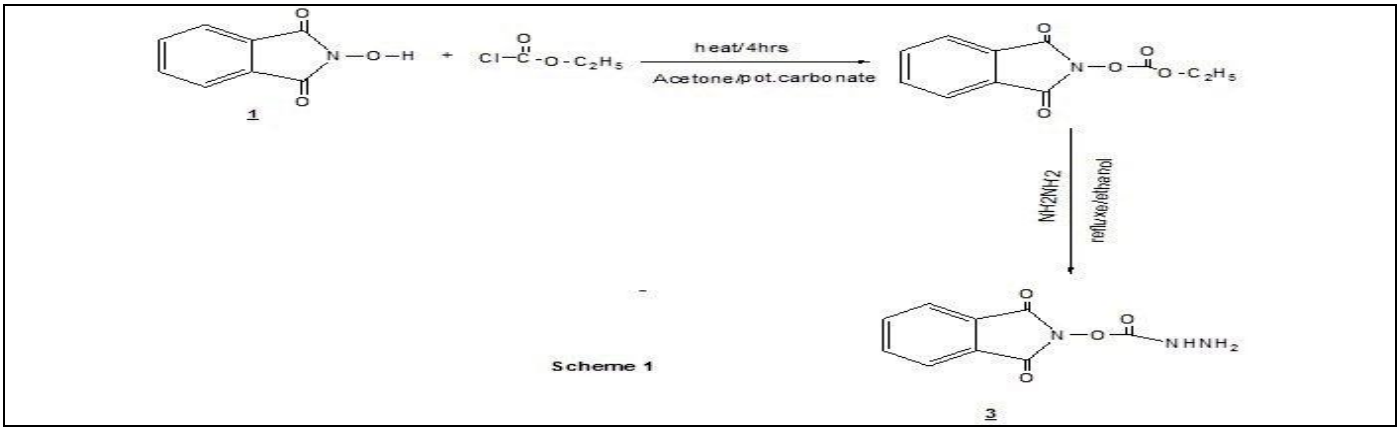
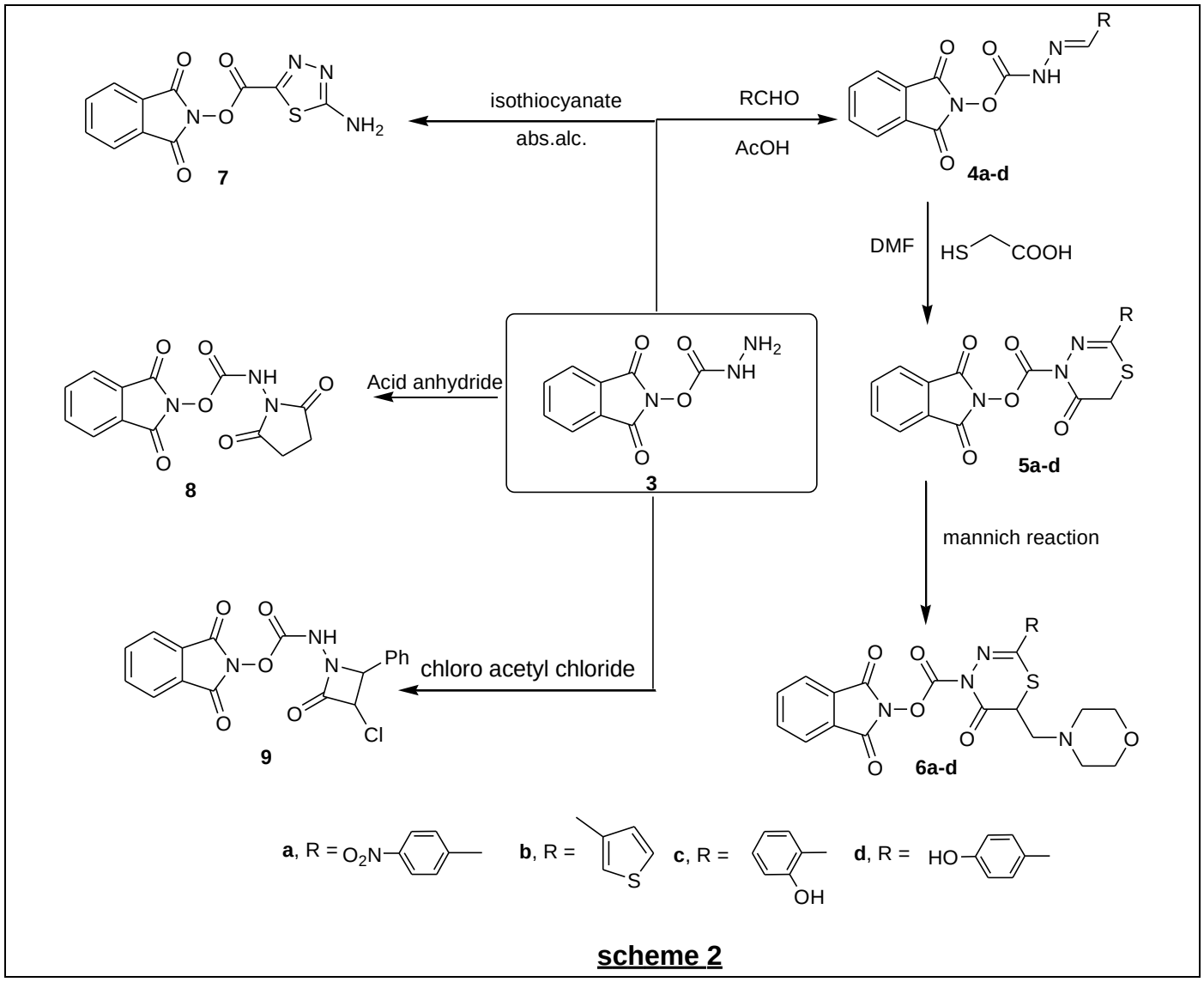



**Fig. 2. Polyploidy as numerical chromosomal aberration induced in cultural cancer spleen cells**




Scheme 1




**scheme 2**

**DISCUSSION**

Genotoxicity tests able to detect drugs that cause genetic damage by interaction with other cellular targets, such as enzymes and microtubules, are particularly interesting because they play a significant role in DNA replication or in segregation of chromosomes during cell division [23].

In the present study, the induction of chromosomal aberrations in spleen cells *in vitro* are in agreement with the observation of [24-26] *in vivo* and *in vitro* [27, 28] who reported the inductions of aneuploidy in mouse oocytes, and Ozkan *et al*. [29] who observed the increase in chromosomal aberrations in mouse bone marrow cells following Taxol administration. Also, the present results are in agreement with investigations on A 549 cells [30] and on human T. lymphocytes [31]. It might be expected that they would induce numerical chromosome aberrations (aneuploidy) [23].

Mitotic aneuploidy may contribute to tumorigenesis by facilitating loss of a chromosome involving tumor suppressor genes that harbor oncogenes [32 and 33]. Chemicals that can interact with the spindle apparatus or interfere with spindle function, preventing normal segregation of chromosomes or chromatids [34], are proven carcinogens [35, 36].

Many studies have indicated that the effectiveness of antitumour compounds in chromosomal aberration induction in cancer rodent cells *in vitro* and *in vivo* [37, 38], can be positively correlated with an *in vivo* tumour response to these agents. This suggests that the chromosomal aberration assay could be used to predict both the sensitivity of human tumour cells to chemotherapeutic agents and the heterogeneity of drug sensitivity within individual tumours [39, 40].

**CONCLUSION**

In conclusion, the correlation between chromosomal aberrations induction, suppression of proliferation rate and antitumour activity observed in this study appeared to further substantiate the validity of the chromosomal aberration assay as a possible method for improving and guiding chemotherapy.

**ACKNOWLEDGEMENT**: None

**CONFLICT OF INTEREST**:

The authors declare that they have no conflict of interest.

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