Eco-friendly one-pot synthesis of some new pyrazolo[1,2-\(b\)]phthalazinediones with antiproliferative efficacy on human hepatic cancer cell lines

Huda R. M. Rashdana, Sobhi M. Gomha b, Marwa S. El-Gendeyc, Maher A. El-Hashashd and Abdel Mohsen M. Soliman

aChemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Giza, Egypt; bDepartment of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt; cDepartment of Chemistry, Faculty of Science (Girls Branch), Al-Azhar University, Cairo, Egypt; dDepartment of Chemistry, Faculty of Science, Ain Shams University, Cairo, Egypt; eTherapeutic Chemistry Department, National Research Centre, Giza, Egypt

ABSTRACT

This work focuses the light on some remarkable achievements in clean and efficient green experimental synthesis, characterization and evaluation of the pharmaceutical and biochemical importance of new series of pyrazolo[1,2-\(b\)]phthalazinediones which synthesized through one-pot three-component condensation reaction of the appropriate of 1,2,3-triazolyl-pyrazole-carbaldehydes with active methylene compounds (as malononitriles or ethyl cyanoacetate) and 6-nitrophthalhydrazide using the grinding method in the presence of sodium hydroxide under solvent-free condition at room temperature, in very excellent yields. All the newly synthesized compounds were characterized by physical and chemical tools (FT-IR, \(1^H\) NMR and mass spectrometry). In addition, all the new synthesized derivatives were screened for their anticancer activity against hepatic cancer cell lines to evaluate their pharmaceutical importance.

ARTICLE HISTORY

Received 23 December 2017
Accepted 2 May 2018

KEYWORDS

Green chemistry; 1,2,3-triazoles; pyrazoles; phthalhydrazides; anticancer activity; multi-component reactions

Introduction

Identification of novel structures may be used in designing potent, new, less toxic and selective anticancer agents remains a major challenge for medical chemistry researchers. The 1,2,3-triazole-based heterocyclic compounds have been well exploited for the generation of many medicinal scaffolds exhibiting anti-HIV(1), antibacterial (2) inhibitors of galectin-3 (3) activities, etc. Moreover, researchers reported 1,2,3-triazole derivatives that exhibited anticancer activities with excellent IC50 and IG50 (4–10). On the other hand, pyrazole and pyrazole derivatives have drawn great attention of the researchers through the past few decades due to their high therapeutic importance as anti-inflammatory (11), antiallergy (12), antiviral (13), antimicrobial (14), anti-diabetic (15) activities, etc. Moreover, pyrazole prodrugs have also been reported to possess significant anticancer activity (16–20). Also, fused phthalazines are considered as effective therapeutically active compounds; they possess a wide range of pharmaceutical applications such as anticancer (21, 22), anticonvulsant (23, 24), antimicrobial (25), antifungal (26) and anti-inflammatory (27) activities. Hence, the development of new methods for the synthesis of pyrazoles, triazoles and phthalazines continues to be an active area of research. Nevertheless, multi-component reactions were considered as an excellent tool for synthesis of complex heterocycles owing to their advantages of the structure diversity, simpler procedures, intrinsic atom economy, energy saving and reduced waste (28–34).

Moreover, referring to the statement “The best solvent is no solvent” (35) and the growing demand for an efficient and clean methodology represent a challenge of great interest in heterocyclic synthetic
chemistry. The exclusion of solvents in chemical reactions has become one of the main criteria in green chemistry (36, 37). Moreover, the grinding technique is of great interest in synthetic organic chemistry as it is carried out under ecofriendly conditions and in the absence of solvent (38–41). Also, the grinding process is done at room temperature and the reaction time range is from 2 to 5 minutes. So, it contributes to the development of a green strategy for the synthesis of organic derivatives in high yield with simple, fewer waste products, efficient, environmentally and economically benign compared to classical methods.

In the light of the above findings and in continuation of our efforts to synthesize new anticancer compounds (42–48), this work aims to synthesize a new series of 1-(3-(1,2,3-triazol-5-yl)pyrazol-4-yl)-pyrazolo[1,2-b]phthalazine, which have not been reported hitherto in multi-component reactions via ecofriendly methods and evaluate their anticancer activity against hepatic cancer and normal cells.

Results and discussion

Owing to the biochemical and pharmaceutical significance of 1,2,3-triazole and pyrazole rings, our growing interest in this part of the work is to incorporate between 1,2,3-triazole and pyrazole rings. Toda et al. (49) report that some of the exothermic reactions could be accomplished in excellent yield through grinding solids together (or liquid/solid) by using the mortar pestle technique which is known as grindstone chemistry. Reactions begin with grinding, with transfer of very small amounts of energy via friction (see Figure 1). Based on this simple technique, we synthesized a series of hydrazones containing 1,2,3-triazole moiety and utilizing these hydrazones in preparation of 1,2,3-triazolyl-pyrazole-carbaldydes. Thus, condensation of acetyl triazole derivatives 1a–e (50) with phenyl hydrazine in the presence of one drop of glacial acetic acid by the grinding method at room temperature afforded the respective triazolyldihydrazone 2a–e in excellent yields (Scheme 1). The chemical structure of 2a–e was established based on elemental data and also on spectral data (IR, 1HNMR, mass). For example, the 1HNMR spectrum of compound 2a displayed one signal at δ = 11.21 ppm attributed to the NH proton, in addition to the expected signals of the two methyl and aromatic protons. The mass spectral data of all products 2 exhibited in each case a molecular ion peak at the correct molecular weight for the respective compound (see Experimental).

When those hydrazones 2a–e were submitted to Vilsmeier reaction conditions, they cyclized to afford the 1,2,3-triazolyl-pyrazole-carbaldyde derivatives 3a–e through intramolecular cyclization reaction (Scheme 1).

![Figure 1. Appearance of reaction media at different times for compound 2a.](image)

**Scheme 1.** Synthesis of 1,2,3-triazolyl-pyrazole-carbaldydes 3a–e.
The structures of 3a–e were deduced from the microanalytical and spectral data. A respective example, the IR spectrum of 3d exhibited strong absorption band at \( \nu = 1715 \text{ cm}^{-1} \) attributed to the carbonyl of the CHO group. Moreover, the \(^1\)H NMR spectrum of 3d showed the absence of a signal at \( \delta = 11.21 \text{ ppm} \) for the (NH) group and instead there appeared two singlet signals for the two protons of pyrazole-H5 and the CHO group at \( \delta = 9.27 \) and 10.52 ppm, respectively, in addition to the expected signals due to the protons of CH3 and aryl groups (see experimental section). The mass spectra of 3d exhibited a molecular ion peak at \( m/z = 407 \) which is consistent with the assigned chemical structure.

Despite the pharmacological and synthetic importance of pyrazolo[1,2-b]phthalazinedione, several multicomponent strategies have emerged for synthesis of this ring system by cyclo-condensation of phthalhydrazone, aldehydes and active methylene compounds like ethyl cyanoacetate or malononitrile using Et3N as a catalyst. However, nowadays, the usage of a solid basic eco-friendly catalyst has been found to be a comfortable synthetic platform rather than the usage of volatile toxic organic bases. So, herein we used NaOH as the eco-friendly basic catalyst.

Also, in view of the pharmaceutical importance of pyrazolo[1,2-b]phthalazinedione, a modification on the 1-position of pyrazolo[1,2-b]phthalazinedione by 5-(pyrazol-3-yl)-1,2,3-triazole is undertaken to check whether it causes a significant change in the bioactivity of pyrazolo[1,2-b]phthalazinedione. Our aim in the current part of this study is developing new potent bioactive 1-(3-(1,2,3-triazol-5-yl)-pyrazol-4-yl)-pyrazolo[1,2-b]phthalazinedione derivatives 6a–j through multicomponent reaction of pyrazole-4-carbaldehydes 3a–e, malononitrile 4a or ethylcyanoacetate 4b and 2,3-dihydro-6-nitrophthalazine-1,4-dione 5 in the presence of sodium hydroxide in catalytic amount via grinding at room temperature (Scheme 2).

The structures of 6a–j were established via elemental analysis and spectral (IR, \(^1\)H NMR, Mass) data. The IR spectra of compounds 6d, taken as a representative example of the products 6a–e, revealed in each case four bands at \( \nu = 1676, 1680, 2222 \) and \( 3287, 3393 \text{ cm}^{-1} \) which are assigned to the 2-carbonyl, nitrile and –\( \text{NH}_2 \) groups. Also, the \(^1\)HNMR spectra showed in addition to the expected signal assigned for CH3 group, pyrazole-H5 and the aromatic protons, two signals at \( \delta = 5.12 \) and 6.23 ppm assigned for the pyrazole-H1 and \( \text{NH}_2 \) protons.

Otherwise, IR spectrum of 6i, taken as a representative example of the derivatives 6f–j, showed a strong biforked absorption band at \( \nu = 3423, 3387 \text{ cm}^{-1} \) for \( \text{NH}_2 \).
strong stretching absorption band at $\nu = 1720 \text{ cm}^{-1}$ for ester C=O and two stretching absorption bands at $\nu = 1665, 1687 \text{ cm}^{-1}$ attributed to the two carbonyl groups of dione. Its $^1\text{H}$ NMR spectrum showed a triplet signal at 1.29 ppm due to three protons of $\text{CH}_3\text{CH}_2$, singlet signal at 2.52 ppm for three protons of $\text{CH}_3$, quartet signal at 4.34 ppm owing to two protons of $\text{CH}_2\text{CH}_3$, singlet signal at 5.3 ppm for two protons of $\text{NH}_2$, singlet signal at 6.34 ppm for pyrazole-$\text{H1}$, singlet signal at 9.34 ppm attributed to pyrazole-$\text{H5}$ and multiplet signal at 7.15–7.92 ppm due to aromatic hydrogen. In addition, mass spectral data of all new derivatives showed correct molecular ion peaks (see Experimental).

The structure of 6 was further confirmed by an alternative synthetic method. Thus, refluxing of compound 3a with malononitriles in ethanol leads to the formation of 7a. Compound 7a was then reacted with 6-nitro-2,3-dihydropthalazinedione in ethanol in the presence of sodium hydroxide to give a compound identical in all respects (IR, mp and mixed mp) to 6a (Scheme 2; Figure 2).

A plausible mechanism for synthesis of pyrazolo[1,2-b]phthalazinedione derivatives 6a–j was illustrated in Scheme 3. Hetarylidene-nitrile derivatives 7a–j were firstly formed from condensation of aldehydes 3a–e and active methylene derivatives 4a,b which on subsequent Michael addition of the NH group of 2,3-dihydro1,4-phthalazinedione 5 to the C=C bond of heterylidenenitrile 7, followed by cyclization then tautomerization, gives the corresponding pyrazolo[1,2-b]phthalazine-5,10-dione derivatives 6a–j.

**Pharmacology**

**Antiproliferative activities**

The *in vitro* growth inhibition activities of the newly synthesized compounds 2a–e, 3a–e and 6a–j were examined against human hepatocellular carcinoma (HepG-2) comparing with the well-known anticancer standard (Doxorubicin) under the same condition by using colorimetric MTT assay. Data generated are used to plot a
dose–response curve in which the concentration of tested compounds required to kill half of the cell population (IC50) was determined. IC50 values were calculated for each experiment separately and mean values ± SD are represented in Table 1. Each compound at each concentration was tested in triplicate in a single experiment, which was repeated three to five times. The results of the studies on antiproliferative activities of tested compounds are summarized in Table 1 and Figure 3.

The results of the studies on antiproliferative activity of tested compounds in Table 1 and Figure 1 show that compound 6f revealed the highest antiproliferative activity (IC50 = 3.01 ± 0.21 µg/mL) toward the human hepatic cancer (HepG2) cell line while it is not active against the normal cell line (BALB/3T3). Moreover, compounds (2b, 3b, 3c, 3e, 6a, 6c and 6i) indicated high activity against the HepG2 cell line but they also showed lower activity against the normal cell line BALB/3T3. On the other hand, compound 2c showed lower antiproliferative activity against HepG2 (IC50 = 69.20 µg/mL) but it is not active against BALB/3T3 in the used range of concentration.

From the data of Table 1, we concluded the following structure–activity relationships (SARs):

- The ester group (CO2Et) at position 2 of the pyrazolo[1,2-b]phthalazinediones ring has higher antiproliferative activity than the cyano group (6f (IC50 = 3.01 ± 0.21 µg/mL) > 6a (IC50 = 13.51 ± 4.48 µg/mL).
- 1,2,3-Triazolyl-pyrazolyl-pyrazolo[1,2-b]phthalazinediones 6 has higher activity than 1,2,3-triazolyl-pyrazolyl-carbaldehydes 3 than triazole-hydrazones 2.
- For 1,2,3-triazolyl-pyrazolyl-pyrazolo[1,2-b]phthalazinediones 6: the presence of a nitro group, a chlorine group (electron-withdrawing group) or a methoxy group led to higher antiproliferative activity compared to their analogs without these groups.

### Table 1. Antiproliferative activity of new derivatives 2a–e, 3a–e and 6a–j toward hepatic cancer and normal cell lines expressed as IC50 values (µg/mL) ± standard deviation from three replicates.

| Cpd. no. | HepG2 (µg/mL) ± SD | BALAB/3T3 (µg/mL) |
|----------|-------------------|--------------------|
| Doxorubicin | 3.56 ± 0.46 | Nd |
| 2a       | Nd                | Nd |
| 2b       | 53.32 ± 11.83     | 64.09 ± 12.00 |
| 2c       | 69.20 ± 9.37      | Nd |
| 2d       | Nd                | Nd |
| 3a       | Nd                | Nd |
| 3b       | 37.52 ± 6.81      | 51.54 ± 6.35 |
| 3c       | 53.24 ± 8.65      | 76.72 ± 4.14 |
| 3d       | Nd                | Nd |
| 3e       | 11.93 ± 3.08      | 39.85 ± 1.17 |
| 6a       | 13.51 ± 4.48      | 18.24 ± 3.61 |
| 6b       | Nd                | Nd |
| 6c       | 22.73 ± 5.36      | 28.34 ± 7.61 |
| 6d       | Nd                | Nd |
| 6e       | 3.01 ± 0.21       | Nd |
| 6f       | Nd                | Nd |
| 6g       | Nd                | Nd |
| 6h       | Nd                | Nd |
| 6i       | 26.37 ± 6.17      | 30.04 ± 8.52 |
| 6j       | Nd                | Nd |

Note: Compounds were tested in concentration from 100 to 0.1 µg/mL; Nd: not detected in used concentrations; concentration of DMSO: 1%.

Figure 3. Cytotoxic activities of the most active compounds against HepG-2 cell lines.
group (electron-donating group) at position 4 in the aryl moiety of the triazole ring decrease activity.
• For 1,2,3-triazolyl-pyrazolyl-carbaldehydes 3: 3e (substituted with the NO₂ group) > 3b (substituted with the Cl group) > 3c (substituted with the OCH₃ group).

**Experimental**

All melting points were determined on an electrothermal apparatus and are uncorrected. IR spectra were recorded (KBr discs) on a Shimadzu FT-IR 8201 PC spectrophotometer. ¹H NMR spectra were recorded in (DCl)SO solutions on a JNM-LA 400 FT-NMR system spectrometer and chemical shifts are expressed in δ ppm units using TMS as an internal reference. Mass spectra were recorded on a GC-MS apparatus and are uncorrected. IR spectra were recorded out at the microanalytical center of Cairo University.

**Chemistry**

**Synthesis of 1-(1-(5-Methyl-1-aryl-1H-1,2,3-triazol-4-yl)ethylidene)-2-phenylhydrazines 2a–e**

A mixture of the appropriate acetyl triazole derivatives 1a–e (10 mmol) and phenyl hydrazine (10 mmol) was ground in a mortar at room temperature, in the presence of drops of acetic acid (2 mmol), for 10–20 minutes. The reaction mixture was poured into water and the solid product was collected by filtration followed by washing with ethanol. The crude product was then recrystallized from acetic acid to give the corresponding hydrazone derivatives 2a–e. The products 2a–e together with their physical constants are listed below.

**1-(1-(5-Methyl-1-phenyl-1H-1,2,3-triazol-4-yl)ethylidene)-2-phenylhydrazine (2a)**

White crystals; yield: 95%; m.p. 161°C; FT-IR (KBr, cm⁻¹): ν 2973 (C–H), 1602 (C=C), 1270 (C–C), 737–752 (m, 9H, Ar-H). MS: M/z [%]: 307 (M⁺, 100), 151 (43), 77 (74), 50 (45). Analysis: calcd. for C₁₇H₁₅N₅ (291): C, 60.71; H, 4.79; N, 24.99%. found: C, 60.82; H, 4.87; N, 24.95%.

**1-(1-(1-(5-Methyl-1-phenyl-1H-1,2,3-triazol-4-yl)ethylidene)-2-phenylhydrazine (2b)**

White crystals; yield: 90%; m.p. 125–127°C; FT-IR (KBr, cm⁻¹): ν 2976 (C–H), 1602 (C=C), 1277 (C–C), 736–750 (m, 9H, Ar-H). MS: M/z [%]: 327 (M⁺, 100), 150 (43), 77 (75), 50 (45). Analysis: calcd. For C₁₇H₁₆ClN₅ (325): C, 62.67; H, 4.95; N, 21.50%. found: C, 62.82; H, 4.87; N, 21.65%.

**1-(1-(1-(4-Chlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)ethylidene)-2-phenylhydrazine (2c)**

White crystals; yield: 93%; m.p. 140–142°C; FT-IR (KBr, cm⁻¹): ν 2973 (C–H), 1602 (C=C), 1277 (C–C), 737–752 (m, 9H, Ar-H), 11.02 (br s, 1H, NH). MS: M/z [%]: 307 (M⁺, 100), 151 (43), 77 (75), 50 (45). Analysis: calcd. for C₁₇H₁₆ClN₅ (307): C, 59.95; H, 4.87; N, 24.95%. found: C, 60.12; H, 4.87; N, 24.95%.

**1-(1-(1-(4-Methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl)ethylidene)-2-phenylhydrazine (2d)**

White crystals; yield: 90%; m.p. 149–151°C; FT-IR (KBr, cm⁻¹): ν 2973 (C–H), 1602 (C=C), 1277 (C–C), 736–750 (m, 9H, Ar-H), 11.02 (br s, 1H, NH). MS: M/z [%]: 327 (M⁺, 100), 151 (43), 77 (75), 50 (45). Analysis: calcd. for C₁₇H₁₆OClN₅ (327): C, 62.82; H, 4.87; N, 24.95%. found: C, 62.95; H, 4.87; N, 24.95%.

**1-(1-(1-(4-Bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)ethylidene)-2-phenylhydrazine (2e)**

White crystals; yield: 90%; m.p. 125–127°C; FT-IR (KBr, cm⁻¹): ν 2973 (C–H), 1602 (C=C), 1277 (C–C), 736–750 (m, 9H, Ar-H), 11.02 (br s, 1H, NH). MS: M/z [%]: 347 (M⁺, 100), 151 (43), 77 (75), 50 (45). Analysis: calcd. for C₁₇H₁₆BrN₅ (347): C, 59.15; H, 4.87; N, 24.95%. found: C, 59.27; H, 4.87; N, 24.95%.

**Synthesis of pyrazoles 3a–e**

Phosphorus oxychloride (20 mL, 20 mmol) was added dropwise with stirring to dimethylformamide (150 mL) at 0–5°C. Then the appropriate hydrazide 2a–e (20 mmol) was added portion-wise with continuous stirring, left overnight at room temperature, poured onto ice-cold water and neutralized with ammonium hydroxide solution (5%). The formed precipitate was filtered, dried and recrystallized from acetic acid to give the corresponding pyrazole derivatives 3a–e, respectively. The products 3a–e together with their physical constants are listed below.

**3-(5-Methyl-1-phenyl-1H-1,2,3-triazol-4-yl)pyrazole-4-carbaldehyde (3a)**

Off-white crystals; yield: 89%; m.p. 186°C; FT-IR (KBr, cm⁻¹): ν 2976, 2973 (C–H), 1718 (C=O), 1630 (C=N), 1595 (C=N), 1 H NMR (400 MHz, DMSO-d₆): δ 2.52 (s, 3H, CH₃), 7.32–7.89 (m, 10H, Ar-H). 9.25 (s, 1H, pyrazole-H5). 10.59 (s, 1H, CHO). 13C-NMR (100 MHz, DMSO-d₆): δ 14.4 (CH₃), 107.0, 120.2, 123.1, 126.3, 127.5, 128.9.
3-(5-Methyl-1-p-chloro-1H,1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (3b)

Off-white crystals; yield: 89%; m.p. 192–194°C; FT-IR (KBr, cm⁻¹): ν 2985, 2970 (C=H), 1715 (C=O), 1630 (C=N), 1600 (C=C); ¹H NMR (400 MHz, DMSO-d₆): δ 2.52 (s, 3H, CH₃), 7.32–7.65 (m, 9H, Ar-H), 9.27 (s, 1H, pyrazole-HS), 10.52 (s, 1H, CHO); MS: M/z [%]: 363 (M⁺, 19), 328 (26), 289 (19), 261 (13), 190 (17), 176 (100), 165 (50), 77 (12), 65 (90). Analysis: calcd. for C₁₉H₁₃N₅O₂ (363): C, 62.73; H, 3.88; N, 19.25%. found: C, 62.89; H, 3.75; N, 19.15%.

3-(5-Methyl-1-p-chloro-1H,1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde (3b)

General procedure for synthesis of pyrazolo[1,2-b]phthalamine derivatives 6a–j

A mixture of pyrazole-4-carbaldehydes 3a–e (5 mmol), malononitrile (4a) or ethylcyanoacetate (4b) (5 mmol), and 6-nitro-2,3-dihydrophthalazine-1,4-dione (5) (1.035g, 5 mmol) was taken in a mortar at room temperature. Solid sodium hydroxide (2 mmol) was added to few drops of water. The reaction mixture was ground by the pestle, under the hood, for 20–30 minutes (monitored through TLC). The reaction mixture was then poured into 2N HCl, and the solid product was collected by filtration followed by washing with water and EtOH. The crude product was recrystallized from ethanol to obtain the pure 6a-j. The products 2a-e together with their physical constants are listed below.

3-Amino-1-(3-(5-methyl-1-phenyl-1H,1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (6a)

Orange crystals; yield: 88%; m.p. 232–234°C; FT-IR (KBr, cm⁻¹): ν 3423, 3364 (NH₃), 2202 (CN), 1695, 1680 (C=O); ¹H NMR (400 MHz, DMSO-d₆): δ 2.52 (s, 3H, CH₃), 5.16 (s, 2H, NH₂), 6.36 (s, 1H, CH), 7.24–8.02 (m, 13H, Ar-H), 9.53 (s, 1H, pyrazole-HS); ¹C NMR (100 MHz, DMSO-d₆): δ 14.6 (CH₃), 52.2 (CH), 69.4 (C-CN), 113.7 (CN), 120.2, 120.8, 122.5, 124.6, 124.9, 126.3, 126.8, 128.5, 128.8, 129.4, 129.9, 132.3, 134.0, 135.2, 139.7, 140.4, 144.1, 149.2, 155.6 (Ar-C and C=N), 162.7, 163.5 (C=O); MS: M/z [%]: 584 (M⁺, 12), 580 (3), 569 (10), 550 (9), 398 (3), 298 (5), 287 (24), 260 (3), 216 (6), 176 (100). Analysis: calcd. for C₃₀H₂₀N₁₀O₄ (584): C, 61.64; H, 3.45; N, 23.96%. found: C, 61.76; H, 3.32; N, 23.89%.

3-Amino-1-(3-(5-methyl-1-phenyl-1H,1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (6b)

Brownish red crystals; yield: 82%; m.p. 257–259°C; FT-IR (KBr, cm⁻¹): ν 3397, 3375 (NH₃), 2197 (CN), 1680, 1665 (2C=O); ¹H NMR (400 MHz, DMSO-d₆): δ 2.52 (s, 3H, CH₃), 5.19 (s, 2H, NH₂), 6.56 (s, 1H, CH), 7.34–8.22 (m, 12H, Ar-H), 9.52 (s, 1H, pyrazole-HS); MS: M/z [%]: 618 (M⁺, 2), 585 (29), 558 (18), 532 (5), 452 (36), 395 (11), 296 (53), 195 (15), 77 (100). Analysis: calcd. for C₁₉H₁₃N₁₀ClO₄ (618): C, 58.21; H, 3.09; N, 22.63%. found: C, 58.12; H, 3.01; N, 22.52%.

3-Amino-1-(3-(1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazole-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (6c)

Brownish red crystals; yield: 73%; m.p. 240–242°C; FT-IR (KBr, cm⁻¹): ν 3395, 3379 (NH₃), 2206 (CN), 1685, 1665
(2C=O); \textit{1}H NMR (400 MHz, DMSO-\textit{d}_6): \delta 2.52 (s, 3H, \textit{CH}_3),
3.85 (s, 3H, OCH_3), 5.09 (s, 2H, NH_2), 6.56 (s, 1H, CH), 7.24–7.72 (m, 12H, Ar-H), 9.52 (s, 1H, pyrazole-H5); MS: M/z [%]: 616 (M^+ +2, 12), 600 (22), 490 (13), 455 (31), 409 (5), 299 (55), 276 (4.03), 267 (10), 77 (100). Analysis: calcd. for C_{31}H_{23}N_{10}O_{5}: C, 59.91; H, 4.11; N, 19.05%. found: C, 59.96; H, 4.03; N, 18.92%.

3-Amino-1-(3-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]pyrazaline-2-carboxilate (6f)

Yellow crystals; yield: 82%; m.p. 217–220°C; FT-IR (KBr, cm^{-1}): \nu 3343, 3335 (NH_2), 1715, 1682, 1665 (3C=O); \textit{1}H NMR (400 MHz, DMSO-\textit{d}_6): \delta 1.24 (t, 3H, \textit{CH}_3CH_2), 2.52 (s, 3H, CH_3), 4.1 (q, 2H, CH_2CH_3), 5.3 (s, 2H, NH_2), 6.34 (s, 1H, CH), 7.34–7.98 (m, 13H, Ar-H), 9.52 (s, 1H, pyrazole-H5); \textit{1}C-NMR (100MHz, DMSO-\textit{d}_6): \delta 14.6 (CH_3), 51.4 (CH), 89.3 (C-COOEt), 117.9, 120.0, 121.6, 122.0, 122.7, 124.7, 126.8, 128.1, 128.9, 129.2, 129.8, 130.4, 131.9, 135.4, 138.1, 140.6, 143.0, 147.4, 150.7, 152.9 (Ar-C and C=N), 163.9, 164.7, 169.5 (C=O); MS: M/z [%]: 631 (M^+, 1), 616 (12), 597 (37), 550 (9), 501 (9), 497 (6), 392 (65), 323 (7), 277 (5), 77 (100), 50 (49). Analysis: calcd. for C_{32}H_{25}N_{10}O_{6}: C, 60.85; H, 3.99; N, 19.96%. found: C, 60.78; H, 19.87; N, 19.91%.
CH₂CH₃), 2.54 (s, 3H, CH₃), 4.42 (q, 2 H, CH₂CH₃), 5.10 (s, 2H, NH₂), 7.55–8.02 (m, 12H, Ar-H), 9.53 (s, 1H, pyrazole-H5); MS: M/z [%]: 676 (M+, 2), 602 (26), 560 (57), 549 (19), 339 (9), 202 (25), 65 (100), 55 (45).

Analysis: calcd. for C₃₂H₂₄N₁₀O₈ (676): C, 56.81; H, 3.58; N, 20.70%. found: C, 56.89; H, 3.69; N, 20.78%.

Alternate synthesis of 6a

(1) Synthesis of 2-((3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)malononitrile (7a).

NaOH (0.116 g, 2 mmol) was added to a mixture of pyrazole-4-carbaldehyde 3a (0.658 g, 2 mmol) and malononitrile (0.132 g, 2 mmol) in ethanol (10 mL). The formed solid after cooling was filtered and recrystallized with ethanol to obtain pure 7a as yellowish crystals; yield: 93%; m.p. 148–150°C; FT-IR (KBr, cm⁻¹): v 2927 (C–H), 2204, 1997 (2CN), 1605 (C=N); ¹H NMR (400 MHz, DMSO-d₆): δ 2.53 (s, 3H, CH₃), 8.29 (s, 1H, =CH), 7.31–7.84 (m, 10H, Ar-H), 9.50 (s, 1H, pyrazole-H5); MS: M/z [%]: 377 (M⁺, 24), 339 (17), 118 (49), 77 (100). Analysis: calcd. for C₂₂H₁₅N₇ (377): C, 70.01; H, 4.01; N, 25.98%. found: C, 70.24; H, 3.95; N, 25.77%.

(1) Reaction of 7a with 6-nitro-2,3-dihydrophthalazine-1,4-dione (5)

Equimolar amounts of 7a (0.377 g, 1 mmol) and 6-nitro-2,3-dihydrophthalazine-1,4-dione (3a) (0.207 g, 1 mmol) in ethanol (10 mL) containing an equivalent amount of NaOH (0.058 g, 1 mmol) was refluxed for 2 h, gave product identical in all respects (m.p., mixed m.p. and IR spectra) with compound 6a.

Pharmacology

Antiproliferative activity

Cells

Cell lines: HepG2 (human hepatic cancer) and BALB/3T3 (murine fibroblast) are being maintained in the Institute of Cancer, Cairo, Egypt. All cancer cell lines were obtained from American Type Culture Collection (Rockville, Maryland, USA) and are being maintained in the Institute of Cancer. HepG2 cells were cultured in Eagle’s Minimum Essential Medium supplemented with 10% FBS; DMEM and RPMI1640 are also alternatives that work well. Aspirate and add fresh culture medium every 2–3 days. HepG2 cell doubling time is 48 h. The BALB/3T3 cell line was cultured in DMEM (Gibco, UK) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (GE Healthcare, Logan, UT, USA). To passage cells, rinse cell monolayer with 1× PBS twice and add pre-warmed (37°C) 0.05% Trypsin-EDTA solution to cover the bottom of the flask; incubate for 5–7 minutes. As cells detach, neutralize the Trypsin by adding 4× volume of complete growth medium with 10% FBS and gently resuspend the cells by pipetting. To avoid clumping do not agitate the cells by shaking the flask while waiting for detachment. Split cells 1:4 every 3 days or 1:8 every 6 days. Cultures should be incubated at 37°C in a humidified atmosphere with 5% CO₂.

Compounds

All compounds were dissolved in DMSO (stock solution 10 mg/mL) and subsequently diluted in culture medium to reach the required concentrations (ranging from 100 to 0.1 µg/mL).

An antiproliferative assay in vitro

Twenty-four hours before addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1 × 10⁴ cells per well. The assay was performed after 72 h exposure to varying concentrations of the tested compounds. The in vitro cytotoxic effect of all compounds was examined using the SRB assay.

Cytotoxic test SRB

The details of this technique have been described by Skehan et al. (51). The cells were attached to the bottom of plastic wells by fixing them with cold 50% TCA (trichloroacetic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 h and then washed five times with tap water. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in 1% acetic acid (POCH, Gliwice, Poland) for 30 minutes. Unbound dye was removed by rinsing (five times) in 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCH, Gliwice, Poland) for determination of the optical density (λ = 540 nm) in a Synergy H4 multimode microplate reader (BioTek Instruments USA).

Conclusions

A new series of pyrazolophtalazinedione derivatives have been successfully developed and characterized, in excellent yields by the grinding method under solvent-free condition at ambient temperature. The newly synthesized compounds seem to be interesting for
pharmaceutical studies. They revealed high potency as anticancer agents. They inhibit the growth of cancer cells but with lower cytotoxic effects on normal cells in the used range of concentrations. The encouraging promising results obtained from anticancer studies on the newly synthesized derivatives make the synthesis of new series of these compounds and studying of their pharmaceutical importance an active area for more and more investigations.

Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

**Dr. Huda R. M. Rashdan** born in 1988 in Giza, Egypt. She graduated from Cairo University, Egypt in 2009 then he carried out her M.Sc. and Ph.D. studies in 2012 and 2015 at Cairo University, Ain Shams University respectively, in the field of organic synthesis. In 2016 she promoted to Researcher in the department of chemistry of natural and microbial products, Pharmaceutical and drug industries division, National Research Center. She published 10 scientific papers all in international journals in the fields of synthetic organic chemistry, chemistry of hydrazonyl halides, chemistry of enaminoxides, green chemistry and bioactive heterocyclic chemistry (There are about 18 citations of her work from 2013 until April 2018 (h-index 2).

**Prof. Sobhi M. Gomha** was born in Fayoum, Egypt. He graduated from Cairo University, Egypt in 1995 then he carried out his M.Sc. and Ph.D. studies in 2002 and 2006 respectively, at Cairo University in the field of organic synthesis. In 2011 he promoted to Associate Professor and in 2016 he was appointed as a full Professor of Organic chemistry at Cairo University. He joined the scientific school of Prof. A. S. Shawali in 1996 and he has published 140 scientific papers and reviews all in international journals in the fields of synthetic organic chemistry, chemistry of hydrazonyl halides, chemistry of enaminoxides, green chemistry and bioactive heterocyclic chemistry (There are about 1420 citations of his work from 2000 until April 2018 (h-index 2).

**Prof. Marwa S. El-Gendey** was born in Giza. She graduated from Al-Azhar University – College of science – chemistry department at 1996 with general score B+ with honors. She received the master degree at 2002 in organic chemistry then the PhD. at 2007 on the same field. She began her functional gradation as lecturer at chemistry department – college of science – Al-Azhar University at 1997 then as an assistant teacher then lecturer at the same department at 2007. She specialized in organic chemistry as she has done several studies on heterocyclic compounds with biotic effects on microorganisms and anti-tumor effects. She now works as an associate professor at Al-Taif University.

**Prof. Maher A. El-Hashash** is an Emeritus Professor of Organic Chemistry, Department of Chemistry, Faculty of Science, Ain Shams University, Giza, Egypt. He graduated with B.Sc. from the Ain Shams University in 1965. He received his M.Sc. and Ph.D. degrees in 1970 and 1972, respectively from the Ain Shams University. He was awarded the degree of Doctor of Science (D.Sc.) from the Ain Shams University after recommendation from after recommendation from a British committee from the Royal Chemical Society in 1999. He has published more than 220 scientific papers and review articles, all in international journals. He supervised 132 M.Sc. and Ph.D. graduate theses. He was invited to present plenary lectures at more than 20 conferences. His research interests are in the fields of 4-aryl-4-oxobut-2-enolic acid, unnatural amino acid, antioxidant heterocycle, 2-marcapto-1-furo-4-yl/1.3-thiazolo/ 1.3-thiazinobenzimidazole, 4-benzimidazo-1-ylpyridazine/oxazine, triazino-benzimidazole and pthalazines.

**Prof. Abdel Mohsen M. Soliman** was born in 1953 in Cairo, Egypt. He graduated from Cairo University, Egypt in 1975. In 1995 he promoted to a full Professor of molecular biology at National Research Center, Egypt. He worked for more than 20 years on molecular biology, immunology, parasitology, drug delivery systems formulations and the biological use of newly synthesized organic compounds, medicinal plants and natural products in protection and treatments of some diseases such as Alzheimer and cancer. He published more than 80 papers in high impact international journals. Member National Committee, Giza, Egypt, 2001-2008.

**ORCID**

Sobhi M. Gomha https://orcid.org/0000-0001-6744-7817

References

[1] San-Félix, A.; Alvarez, R.; Veláezquez, S.; De Clercq, E.; Balzarini, J.; Camarasa, M.J. Nucl. Nucleot. 1995, 14, 595–598.
[2] Wang, X.-L.; Wan, K.; Zhou, C.-H. Eur. J. Med. Chem. 2010, 45, 4631–4639.
[3] Salameh, B.A.; Leffler, H.; Nilsson, U.J. Bioorg. Med. Chem. Lett. 2005, 15, 3344–3346.
[4] Duan, Y.-C.; Zheng, Y.-C.; Li, X.-C.; Wang, M.-M.; Ye, X.-W.; Guan, Y.-Y.; Liu, G.-Z.; Zheng, J.-X.; Liu, H.-M. Eur. J. Med. Chem. 2013, 64, 99–110.
[5] Penthala, N.R.; Madhukuri, L.; Thakkar, S.; Madadi, N.R.; Lamture, G.; Eoff, R.L.; Crooks, P.A. Med. Chem. Commun. 2015, 6, 1535–1543.
[6] Stefely, J.A.; Palchaudhuri, R.; Miller, P.A.; Peterson, R.J.; Moraski, G.C.P.; Hergenrother, P.J.; Miller, M.J. J. Med. Chem. 2010, 53, 3389–3395.
[7] Philip, S.; Purohit, M.N.; La, K.K.; Eswar, M.S.; Raizaday, T.; Prudhvi, S.; Pujar, G.V. Int. J. Pharm. Sci. 2014, 6, 185–189.
[8] Pohkholdylo, N.; Shylya, O.; Matyiuchuk, V. Sci. Pharm. 2013, 81, 663–676.
[9] Ashwiini, N.; Garg, M.; Mohan, C.D.; Fuchs, J.E.; Rangappa, S.; Anusha, S.; Swaroop, T.R.; Rakesh, K.S.; Kanojia, D.; Madan, V.; Bender, A.; Koeffler, H.P.; Rangappa, B.K.S. Bioorg. Med. Chem. 2015, 23, 6157–6165.
[10] Yan, S.-J.; Liu, Y.-J.; Chen, Y.-L.; Liu, L.; Lin, J. Bioorg. Med. Chem. Lett. 2010, 20, 5225–5228.
[11] Sauzem, P.D.; Sant’Anna, G.D.S.; Machado, P.; Duarte, M.M.M.F.; Ferreira, J.; Mello, C.F.; Beck, P.; Bonacorso,
