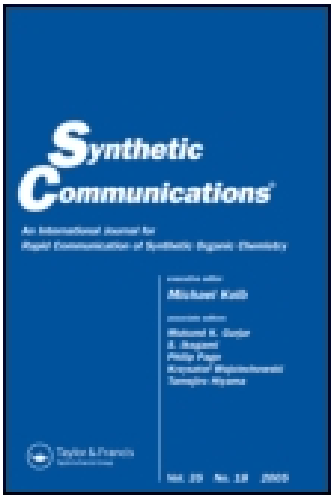


Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

Efficient and Convenient Methods for Synthesis of Some Phthalazine Derivatives and Their Evaluation of Cytotoxicity

Supriti Sen [a], Snehasis Banerjee [b], Susanta S. Adhikari [c], Anuradha Moirangthem [d], Anupam Basu [d] & Pabitra Chattopadhyay [a]

[a] Department of Chemistry, Burdwan University, Golapbag, Burdwan, India

[b] Government College of Engineering and Leather Technology, Kolkata, India

[c] Department of Chemistry, Calcutta University, Kolkata, India

[d] Molecular Biology and Genetics Laboratory, Department of Zoology, Burdwan University, Golapbag, Burdwan, India

Published online: 20 Feb 2014.

To cite this article: Supriti Sen, Snehasis Banerjee, Susanta S. Adhikari, Anuradha Moirangthem, Anupam Basu & Pabitra Chattopadhyay (2014) Efficient and Convenient Methods for Synthesis of Some Phthalazine Derivatives and Their Evaluation of Cytotoxicity, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 44:6, 847-857, DOI: 10.1080/00397911.2013.837486

To link to this article: http://dx.doi.org/10.1080/00397911.2013.837486

Synthetic Communications®, 44: 847–857, 2014

ISSN: 0039-7911 print/1532-2432 online
DOI: 10.1080/00397911.2013.837486

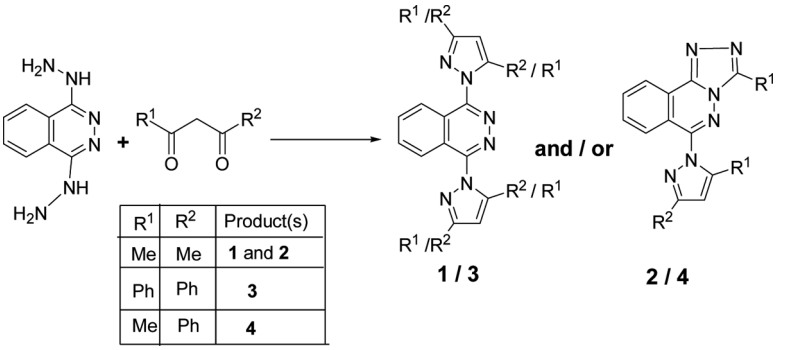
Taylor & Francis
Taylor & Francis Group

EFFICIENT AND CONVENIENT METHODS FOR SYNTHESIS OF SOME PHTHALAZINE DERIVATIVES AND THEIR EVALUATION OF CYTOTOXICITY

Supriti Sen,[1] Snehasis Banerjee,[2] Susanta S. Adhikari,[3] Anuradha Moirangthem,[4] Anupam Basu,[4] and Pabitra Chattopadhyay[1]

[1]*Department of Chemistry, Burdwan University, Golapbag, Burdwan, India*
[2]*Government College of Engineering and Leather Technology, Kolkata, India*
[3]*Department of Chemistry, Calcutta University, Kolkata, India*
[4]*Molecular Biology and Genetics Laboratory, Department of Zoology, Burdwan University, Golapbag, Burdwan, India*

GRAPHICAL ABSTRACT



R^1	R^2	Product(s)
Me	Me	**1** and **2**
Ph	Ph	**3**
Me	Ph	**4**

Abstract *A systematic study of the reaction of 1,4-dihydrazinophthalazine (DHPH) with 1,3-dicarbonyls [*viz.*, acetylacetone (acac), dibenzoylmethane (bzbz), and 1-benzoylacetone (bzac)] varying the reaction conditions was carried out to obtain the phthalazine derivatives (**1**–**4**). One-pot reaction of DHPH with acac led to the formation of two compounds **1** and **2**, with various factors such as the presence of the acid or base, amount of the acac, time of reflux, and the temperature. The reaction conditions of DHPH with bzbz or bzac are sort of different to isolate the products **3** and **4**, respectively. The derivatives (**1**–**4**) have been characterized by elemental analyses, ^{1}H NMR, and electrospray ionization–mass spectrometry (ESIMS) and the cytotoxic activity of the compounds **1**–**4** was evaluated on HeLa cell line.*

[Supplementary materials are available for this article. Go to the publisher's online edition of Synthetic Communications® *for the following free supplemental resource(s): Full experimental and spectral details.]*

Keywords Cytotoxicity; DFT calculation; 1,3-dicarbonyls; phthalazines

Received April 26, 2013.

Address correspondence to Pabitra Chattopadhyay, Department of Chemistry, Burdwan University, Golapbag, Burdwan 713104, India. E-mail: pabitracc@yahoo.com

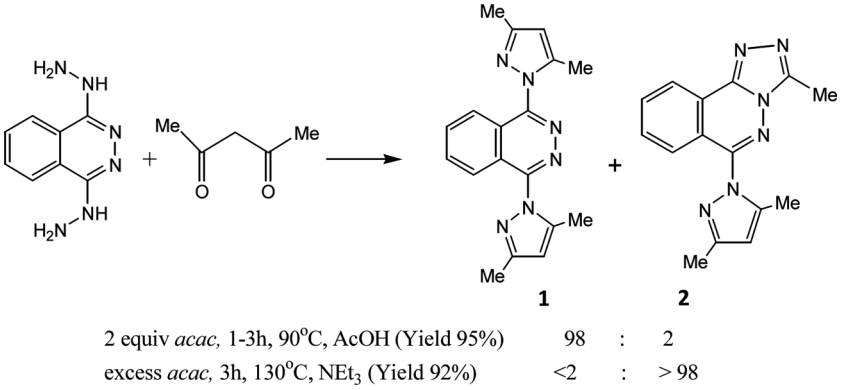
INTRODUCTION

The synthesis of new compounds and testing their biological and pharmacological activities are the major goals of drug development projects. Nitrogen-containing heterocyclic compounds have received much attention as shown by the numerous studies published on their applicability in different areas, especially as drugs.[1,2] Phthalazines are examples of nitrogen heterocycles that possess exciting biological properties such as antimicrobial,[3] anticonvulsant,[4] antifungal,[5] cytotoxic,[6] antitumor,[7–10] antihypertensive,[11,12] antidiabetic,[13,14] anticancer,[15] and anti-inflammatory activities.[16] Therefore, a number of methods have been reported for the synthesis of phthalazine derivatives including the reaction of phthalhydrazide, aromatic aldehydes, and malononitrile,[17,18] the reaction of phthalhydrazide and dialkyl acetylenedicarboxylates in the presence of N-heterocycles,[19] and palladium-catalyzed 1,3-dipolar cycloaddition with phthalhydrazide.[20] However, most of the reported synthetic procedures are tricky and only a sort class of phthalazine derivatives were synthesized. So, the development of new, easy, synthetic methods for the preparation of heterocycles containing phthalazine ring fragments is still challenging. Earlier reports[21] has shown the synthesis of pyrazoles derived from dihydralazine but the yield was too poor, as the reaction mechanism was not established and application of the products was unexplored.

To achieve the good yield of the target phthalazine derivative, here, a simple procedure for the synthesis of some phthalazine derivatives (**1**–**4**) from the reaction of hydrazine with 1,3-dicarbonyl has been explored by monitoring the chemical environments. From the systematic study of reaction, a mechanistic pathway has been proposed and established by the theoretical (DFT) calculations. The products have been characterized using physicochemical and spectroscopic tools. Evaluation of the cytotoxicity compounds (**1**–**4**) on human cancer cell (HeLa) line was carried out, from which it was observed that compound **3** could be a potent anticancer agent.

RESULTS AND DISCUSSION

To achieve the suitable condition we initiated the reaction of 1,4-dihydrazino phthalazines (DHPH) with $MeCOCH_2COMe$ (acac) at different conditions, varying the presence of the acid or base, amount of the acac, time of reflux, and the temperature. Reaction of 1 equivalent 1,4-dihydrazinophthalazine (DHPH) and 2 equivalents of acac in AcOH at 90 °C for 2.0 h resulted in 1,4-bis-(3,5-dimethyl-pyrazol-1-yl)-pthalazine (**1**) (95% total yields with 98% selectivity). The reaction exhibited the transformation from hydrazino to pyrazolyl derivatives under acidic conditions at a comparatively low temperature (shown in Scheme 1). Similar reaction under basic conditions at a higher temperature gave 6-(3,5-dimethyl-pyrazol-1-yl)-3-methyl-[1,2,4]triazolo[3,4-a] phthalazines (**2**) almost quantitatively instead of **1**. Reactions of DHPH and excess amount of acac in Et_3N (2 equivalents) at 130 °C for 3.0 h resulted in **2** (92% total yields with 98% selectivity). The ratio between phthalazines **1** and **2** is dependent upon the pH of medium, amount of acac, time of reflux, and temperature on the basis of the analyses of the results (Table 1). So at low pH values, the carbonyl oxygen atom of the imine intermediate gets protonated and endures nucleophilic attack by adjacent hydrazine-N atom and gives the

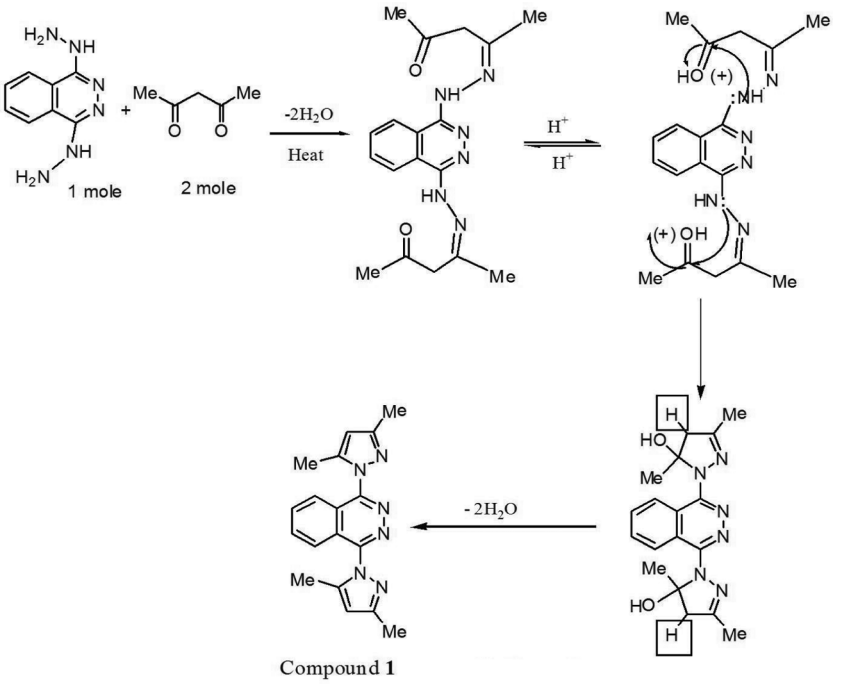


Scheme 1. Synthetic scheme of compounds **1** and **2**.

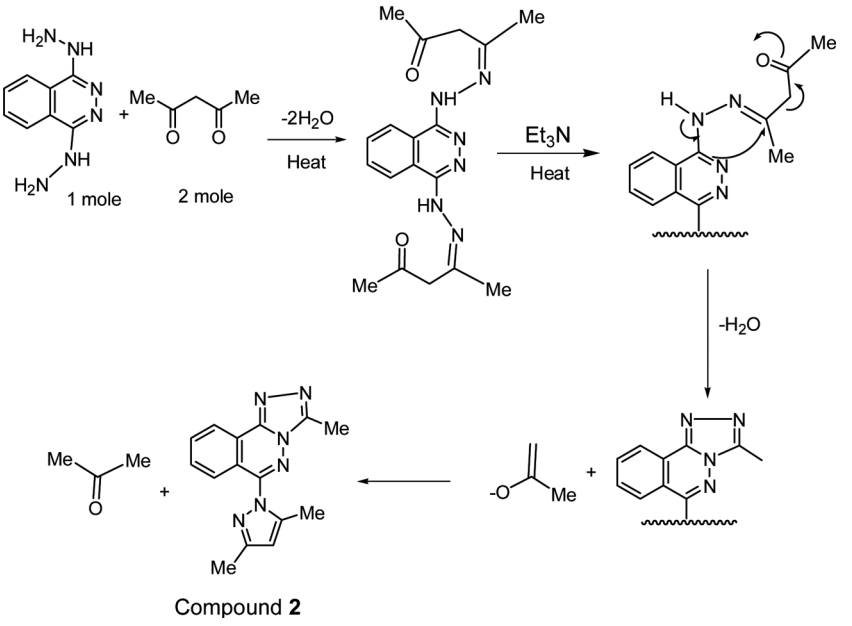
pyrazole ring as product (Scheme 2). Therefore, by the reaction of 1 mol of DHPH and 2 mol of acac in the presence AcOH gives maximum yield of compound **1** with the elimination of four water molecules following pathway 1. On the other hand, at high pH values electrons on the N-atom of the phthalazine ring are available and the adjacent double bond is active for nucleophilic attack giving triazole ring as product (Scheme 3). Consequently by the reaction of DHPH and acac at 1:2 ratio in the presence of Et_3N gives a maximum amount of compound **2** with elimination of three water molecules and one acetone molecules following pathway 2. Moreover, compound **2** is highly thermodynamically stable than **1** (Fig. 1). This is theoretically also proved (Table S1). Reaction path (path 1) is exothermic (ca. −12.79 kcal/mol) for compound **2** while endothermic for compound **1** (ca. +2.16 kcal/mol, path 1). This reaction occurs via the imine intermediate (**a**), which becomes more stable when it is H-bonded (**b**) with two water molecules coming from solvent molecule (Fig. S1). To

Table 1. Dependence of the ratio between products **1** and **2** upon the medium, amount of acac, time of reflux, and temperature

						Product ratio	
Entry	Amount of acac	Time (h)	Temperature (°C)	Medium acidic/basic	Total yield (%)	**1**	**2**
1	2 equiv.	2	90	—	80	94	06
2	Excess	2	90	—	85	92	08
3	Excess	3	110	—	87	31	69
4	Excess	3	130	—	87	20	80
5	2-equiv.	2	90	AcOH	95	98	02
6	Excess	3	90	AcOH	95	96	04
7	Excess	3	130	AcOH	96	80	20
8	Excess	3	130	K_2CO_3	80	50	50
9	Excess	3	130	C_5H_5N	90	06	94
10	2-equiv	2	130	Et_3N	85	<10	>90
11	Excess	3	130	Et_3N	92	<02	>98
12	Excess	3	130	NaH	92	<02	>98



Scheme 2. Pathway 1 for the synthesis of compound **1** in AcOH medium.



Scheme 3. Pathway 2 for the synthesis of compound **2** in Et_3N medium.

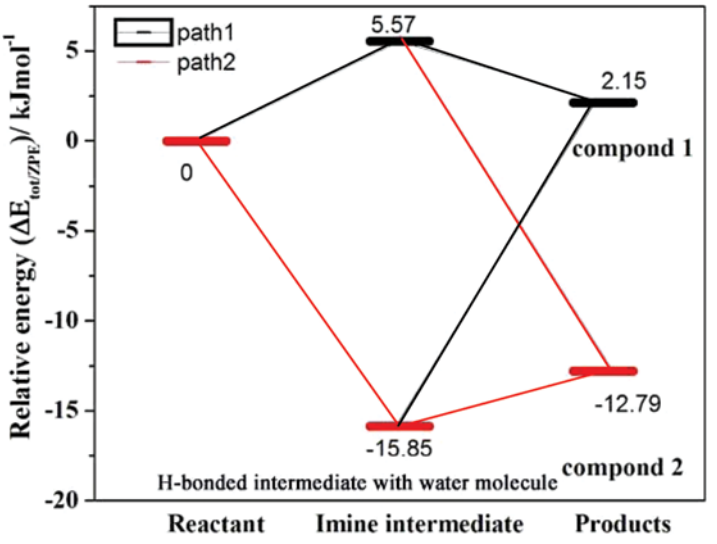
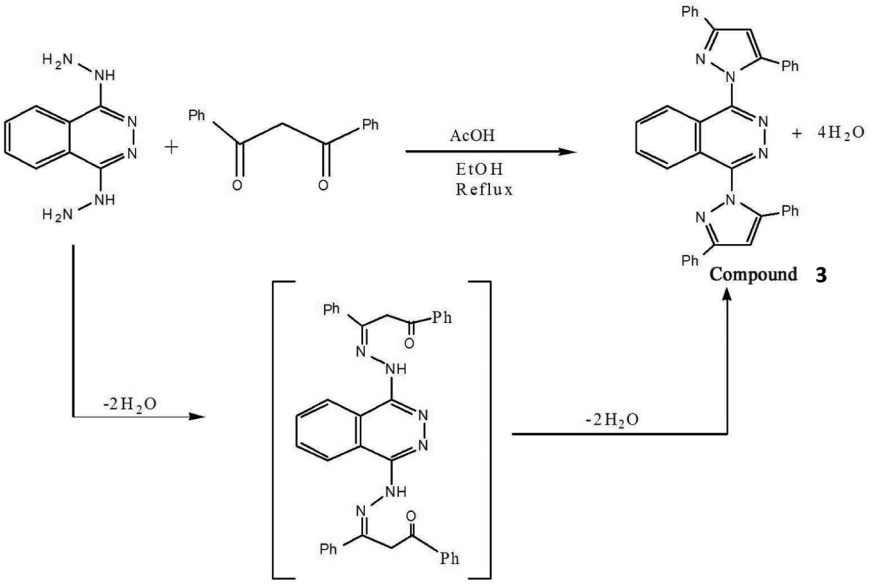


Figure 1. Energy level diagram of reactant, imine intermediate, and products. (Figure is provided in color online.)

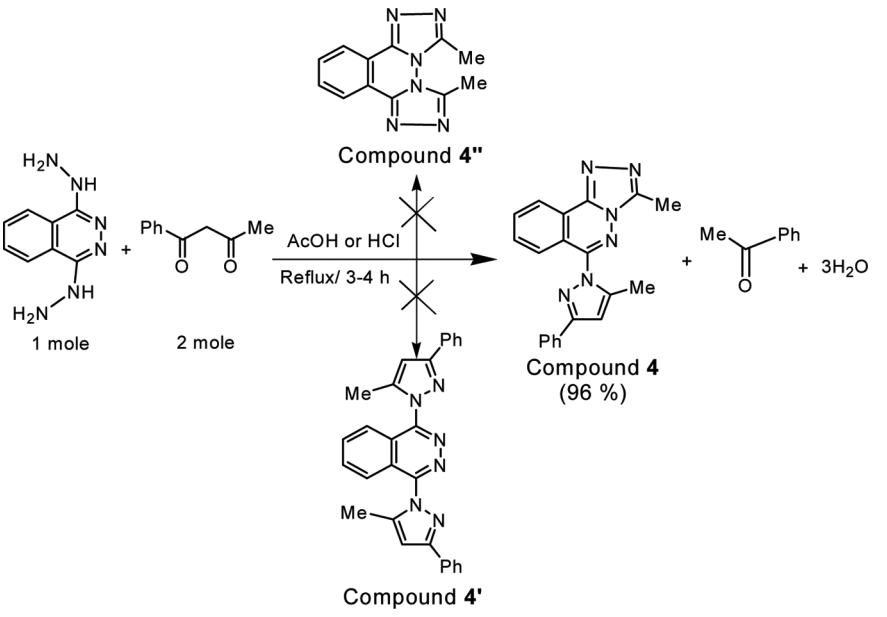
clarify the configurations and energy level of compound **1** and **2**, DFT calculations (Figs. S2 and S3) were carried out in G09W program using B3LYP/6-31G(d) calculation[22] and correlation function[23] as implemented in the Gaussian program package Gaussian 09. Thermal contribution to the energetic properties was considered at 298.15 K and one atmosphere pressure. The energy of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of compound **1** and **2** are −0.2211 eV, −0.0645 eV and −0.2318 eV, −0.0698 eV respectively.

To gain more evidence, the reactions between DHPH with $PhCOCH_2COPh$ (bzbz) and with $PhCOCH_2COMe$ (bzac) were done. The reaction of DHPH and bzbz gave 1,4-bis(3,5-diphenylpyrazolyl)phthalazine **3** in 70% yields (Scheme 4) only at in acidic condition at 130 °C. Logically it had to proceed through an imine intermediate **I-1** prior to the cyclization to form **3**. A similar reaction between DHPH and bzac under acidic conditions at 130 °C produced completely different results (Scheme 5), the almost quantitatively collected product being 3-methyl-6-(5-methyl-3-phenyl-pyrazol-1-yl)-[1,2,4]triazolo[3,4-a]phthalazine (**4**) in which a triazole ring with Me-substituent is fused onto the phthalazines skeleton and only one 3-Me, 5-Ph pyrazole ring is realized. Given the nature of mixed substituents in bzac, the observed selectivity is tremendously high because neither the 5-Me, 3-Ph pyrazole analog 1,4-bis-(5-methyl-3-phenyl-pyrazol-1-yl)-phthalazine (**4′**) nor the analog having triazole ring with Ph-substituent **4″** could be found from the product analysis. It was noted also that the triazole ring fusion is single, not double. The selectivity for the formation of 3-Me, 5-Ph pyrazole could be attributed to the high carbonyl character of the -MeCO group than that of -PhCO group and as a result the imine intermediate **I-1** is preferred over intermediate **I-2** (Fig. S4). From the theoretical calculation it is also reflected that the formation of **I-1** (ca. −15.61 kcal/mol) is energetically more favorable than that of **I-2** (ca. −15.59 kcal/mol) (Fig. S5). The



Scheme 4. Pathway for the synthesis of compound **3**.

selectivity of Me attachment over Ph attachment to the triazole ring could be rationalized by the stability of the correspondent leaving groups, namely, $-CH_2COPh$ vs. $-CH_2COMe$. The $-CH_2COPh$, being more stable carbanion than



Scheme 5. Pathway for the synthesis of compound **4**.

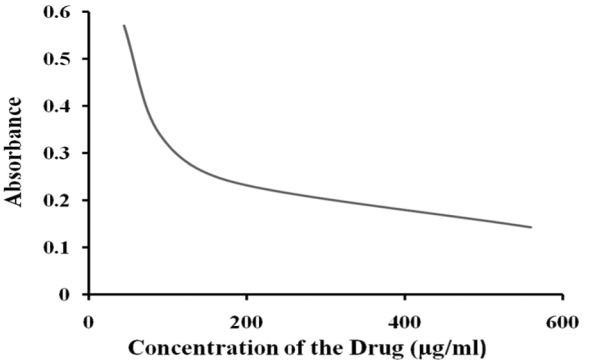


Figure 2. Effect of **3** on HeLa cells by MTT assay. The increasing concentration of the compound shows the increase number of cellular death as evident from the absorbance of developed formazon at 590 nm.

the carbanion -CH_2COMe, is preferred to leaving group one under acidic conditions. Eventwise, the formation of triazole-pyrazole ring fusion is judged to be more stabilized than that of pyrazole-pyrazole ring fusion proved theoretically. The first N-atom that is part of the triazole ring becomes formally positively charged due to an extensive delocalization in the systems, which effectively modifies the polarity of the second N-atom of phthalazines and therefore the second N-atom is much less nucleophilic toward the imine intermediate. As a consequence, the reaction pathway leading to the formation of second triazole ring is not operative, whereas the pathway leading to the formation of pyrazole ring is virtually unaffected.

A cytotoxic compound is generally a good therapeutic agent because it rapidly destroys cancer cells. Cytotoxicity of the compounds was tested on human cervical cancer (HeLa) cell line using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (experimental details in Supporting Information). Here, after incubation of the corresponding compounds (**1**–**4**) and removal of the cultured media, the absorbance of the developed purple color in dimethylsulfoxide (DMSO) having formazon was recorded at 590 nm to measure the cell viability (i.e., number of sustained living cancer cell). From this study, it was found that compound **3** was found to be a good cytotoxic agent against HeLa cell as the measured absorbance was reduced significantly with increase of concentration of compound **3** (Fig. 2), whereas no observable cytotoxic effect was shown by the compounds **1, 2**, and **4** because the absorbance remained constant with concentration of the organic moieties (**1, 2**, and **4**) (Fig. 3). From literature it was seen that most of the phthalazine derivatives show 30% cell viability during the MTT assay experiment. A few number of potential phthalazine derivatives show 60% cell viability, but compound **3** shows 75% cell viability.[24] This observed cytotoxicity of 1,4-bis-(3,5-diphenyl-pyrazol-1-yl)-phthalazines is due to the high lipophilicity of **3**, which facilitate the increased uptake across the lipid cell membrane to destroy the carcinogenic cells to be eligible as a potent anticancer candidate by apoptosis assay. It is similar to the previous reports of apoptosis in breast cancer cells by different pthalazine class compounds.[25]

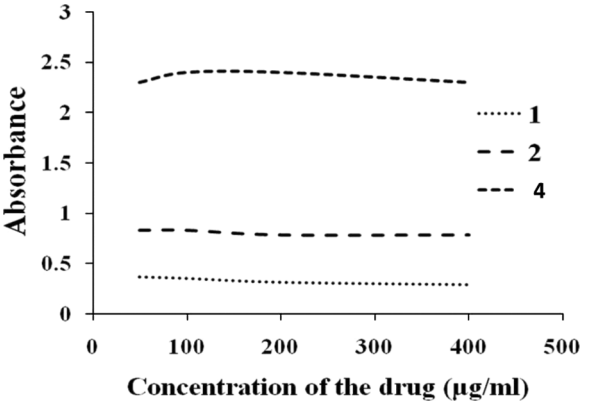


Figure 3. No observable cytotoxic effect of the drugs **1, 2**, and **4** as measured by MTT assay. With the increasing concentration of the drug there was no change in the absorbance of the developed formazon as read spectrophotometrically at 590 nm.

EXPERIMENTAL

All the reagents were obtained from commercial sources and used without further purification. The ^{1}H NMR spectra were obtained on a Bruker AC500 spectrometer with chemical shifts reported in δ values relative to the residual solvent resonance of $CDCl_3$. Electrospray ionization (ESI) mass spectra were recorded on a Qtof Micro YA263 mass spectrometer and the mp were recorded by Fargo melting-point apparatus MP-2D. 1,4-Dihydrazinophthalazine was synthesized following the literature procedure.[26]

Preparation of 1 and 2

A mixture of DHPH (0.01 mol) and acac (0.02 mol or more) was heated in the presence of respective medium on oil bath and cooled. The residue was dissolved in dichloromethane (DCM) and filtered through cellite. The solvent of the filtrate was removed completely by rotary evaporation and a mixed solvent of ether–hexane (1:1) was added into the round-bottom flask to get the solid compound by rubbing with a spatula. Recrystallized product was obtained from ethyl acetate solution.

1,4-Bis-(3,5-dimethyl-pyrazol-1-yl)-pthalazine (1). $C_{18}H_{18}N_6$. Anal. found: C, 67.92; H, 5.66; N, 26.41. Calc.: C, 67.37, H, 5.63, N, 26.38. Mp $223 \pm 1\,^{\circ}C$; ESI-MS: $[M+H]^+$, m/z, 319. 12 and $[M+Na]^+$, m/z, 341.09; ^{1}H NMR (δ, ppm in $CDCl_3$): 8.39 (m, 2H), 7.96 (m, 2H), 6.16 (s, 2H), 2.45 (s, 6H), 2.38 (s, 6H) (Figs. S6 and S7). Yield: 90%.

6-(3,5-Dimethyl-pyrazol-1-yl)-3-methyl-[1,2,4]triazolo[3,4-a] phthalazines (2). $C_{15}H_{14}N_6$. Anal. found: C, 64.74; H, 5.63; N, 30. 21. Calc.: C, 63.96, H, 5.03, N, 29.95. Mp $217 \pm 1\,^{\circ}C$; ESI-MS: $[M+H]^+$, m/z, 279.10 and $[M+Na]^+$, m/z, 301.07; ^{1}H NMR (δ, ppm in $CDCl_3$): 8.71 (d, 1H), 8.05 (d, 1H), 7.97 (q, 1H), 7.76

(q, 1H), 6.16 (s, 1H), 2.80 (s, 3H), 2.39 (s, 3H), 2.37 (s, 3H) (Figs. S8 and S9). Yield: 90%.

Preparation of 3 and 4

A mixture of DHPH (0.01 mol) and bzbz or bzac (0.02 mol) in EtOH was refluxed for 3 h. The ethanol was then evaporated off by rotary evaporator, and DCM was added followed by filtration through cellite. The DCM was removed completely using rotary evaporator and then ether was added to get the solid product. Recrystallized product was obtained from DCM–hexane mixture.

1,4-Bis(3,5-diphenylpyrazolyl)phthalazine (3). $C_{38}H_{26}N_6$. Anal. found: C, 80.56; H, 4.59; N, 14.84. Calc.: C, 79.98; H, 4.48; N, 14.16. Mp $231 \pm 1\,^{\circ}C$; ESI-MS: $[M+H]^+$, m/z, 567.12 and $[M+Na]^+$, m/z, 589.08; ^1H NMR (δ, ppm in $CDCl_3$): 8.40 (m, 2H); 8.03 (m, 2H), 7.94 (m, 4H), 7.48 (t, 6H), 7.40 (t, 3H), 7.27 (m, 10H) (Figs. S10 and S11). Yield: 90%.

3-Methyl-6-(5-methyl-3-phenyl-pyrazol-1-yl)-[1,2,4]triazolo[3, 4-a]phthalazine (4). $C_{20}H_{16}N_6$. Anal. found: C, 70.58; H, 4.70; N, 24.86. Calc.: C, 69.68; H, 4.60; N, 24.70. Mp $181 \pm 1\,^{\circ}C$; ESI-MS: $[M+H]^+$, m/z, 341. 09 and $[M+Na]^+$, m/z, 363.09; ^1H NMR (δ, ppm in $CDCl_3$): 8.29 (d, 1H), 7.59 (d, 1H), 7.57 (t, 1H), 7.35 (t, 1H), 7.28 (m, 5H), 5.29 (s, 1H), 2.43 (s, 3H), 2.22 (s, 3H); (Figs. S12 and S13). Yield: 90%.

CONCLUSION

Reactions of DHPH with 1,3-dicarbonyls in different conditions have been carried out to obtain the tagtted pyrazole and triazole pyrazole and triazole derivatives of hydrazine, quantitatively. All the products have been well characterized. Plausible mechanisms supported by theoretical calculations (by DFT, density functional theory) for the reaction of facile synthesis of products have been proposed. The synthesized compounds (**1**–**4**) were screened against HeLa (cervical) cell to check their anticancer activity, and among them the compound **3** was found to be active against human cervical cancer cell HeLa. It has an ample scope for further study in developing this compound as an anticancer agent.

SUPPLEMENTAL MATERIALS

Go to the publisher's online edition of *Synthetic Communications*® to view the free supplemental file.

ACKNOWLEDGMENTS

Financial assistance from the Council of Scientific & Industrial Research (CSIR), New Delhi, India, is gratefully acknowledged. A.M. and A.B. are thankful to DBT, New Delhi for biological study.

REFERENCES

1. Chorghade, M. S. *In Drug Discovery and Development*; John Wiley & Sons: Hoboken, NJ, 2007, Vol. 2.
2. Lednicer, D.; Mitscher, L. A. In *The Organic Chemistry of Drug Synthesis*; John Wiley & Sons: New York, 1977; Vol. 1.
3. Singh, S.; Yadav, A.; Meena, A. K. Pharmacological action and SAR of phthalazine derivatives: A review. *Internat. J. Chem. Anal. Sci.* **2010**, *1*, 79–87.
4. Grasso, S.; Desarro, G.; Micale, N.; Zappala, M.; Puia, G.; Baraldi, M.; Demicheli, C. Synthesis and anticonvulsant activity of novel and potent 6,7-methylenedioxyphthalazin-1(2-*H*)-ones. *J. Med. Chem.* **2000**, *43*, 2851–2859.
5. Ryu, C.-K.; Park, R.-E.; Ma, M.-Y.; Nho, J.-H. Synthesis and antifungal activity of 6-arylamino-phthalazine-5,8-diones and 6,7-bis(arylthio)-phthalazine-5,8-diones. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2577–2580.
6. Kim, J. S.; Rhee, H. K.; Park, H. J.; Lee, S. K.; Lee, C. O.; Park, C. H. Y. Synthesis of 1-/2-substituted-[1,2,3]triazolo[4,5-*g*]phthalazine-4,9-diones and evaluation of their cytotoxicity and topoisomerase II inhibition. *Bioorg. Med. Chem.* **2008**, *16*, 4545–4550.
7. Cockcroft, X.; Dillon, K. J.; Dixon, L.; Drzewiecki, J.; Eversley, P.; Gomes, S.; Hoare, J.; Kirrigan, F.; Natthews, I.; Menear, K. A.; Martin, N. M. B.; Newton, R.; Paul, J.; Smith, G. C. M.; Vile, J.; Whittlr, A. Phthalazinones 2: Optimisation and synthesis of novel potent inhibitors of poly(ADP-ribose)polymerase. *J. Bioorg. Med. Chem. Lett.* **2006**, *16*, 1040–1044.
8. Cockcroft, X.; Dillon, K. J.; Dixon, L.; Drzewiecki, J.; Kirrigan, F.; Loh, V. M.; Martin, N. M. B.; Menear, K. A.; Smith, G. C. M. Phthalazinones, part 1: The design and synthesis of a novel series of potent inhibitors of poly(ADP-ribose)polymerase. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2235–2238.
9. Kim, J. S.; Lee, H.; Suh, M.; Choo, H. P.; Lee, S. K.; Park, H. J.; Kim, C.; Park, S. W.; Lee, C. Synthesis and cytotoxicity of 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione derivatives. *Bioorg. Med. Chem.* **2004**, *12*, 3683–3686.
10. Haikal, A.; El-Ashery, E.; Banoub, J. Synthesis and structural characterization of 1-(D-glycosyloxy)phthalazines. *Carbohydr. Res.* **2003**, *338*, 2291–2299.
11. Demirayak, S.; Karaburun, A.; Beis, R. Some pyrrole substituted aryl pyridazinone and phthalazinone derivatives and their antihypertensive activities. *Eur. J. Med. Chem.* **2004**, *39*, 1089–1095.
12. Watanabe, N.; Kabasawa, Y.; Takase, Y.; Matsukura, M.; Miyazaki, K.; Ishihara, H.; Kodama, K.; Adashi, H. 4-Benzylamino-1-chloro-6-substituted phthalazines: Synthesis and inhibitory activity toward phosphodiesterase 5. *J. Med. Chem.* **1998**, *41*, 3367–3372.
13. Madhavan, G. R.; Chakrabarti, R.; Kumar, S. K.; Misra, P.; Mamidi, R. N.; Balraju, V.; Ksiram, K.; Babu, R. K.; Suresh, J.; Lohray, B. B.; Lohray, V. B.; Iqbal, J.; Rajagopalan, R. Novel phthalazinone and benzoxazinone containing thiazolidinediones as antidiabetic and hypolipidemic agents. *Eur. J. Med. Chem.* **2001**, *36*, 627–637.
14. Lenz, E. M.; Wilson, I. D.; Wright, B.; Partidge, E. A.; Roddgers, C. T.; Haycock, P. R.; Lindon, J. C.; Nicholson, J. K. A comparison of quantitative NMR and radiolabelling studies of the metabolism and excretion of Statil (3-(4-bromo-2-fluorobenzyl)-4-oxo-3H-phthalazin-1-ylacetic acid) in the rat. *J. Pharm. Biomed. Anal.* **2002**, *28*, 31–43.
15. Carling, R. W.; Moore, K. W.; Street, L. J.; Wild, D.; Isted, C.; Leeson, P. D.; Thomas, S.; O'Connor, D.; McKernan, R. M.; Quirk, K.; Cook, S. M.; Atack, J. R.; Wafford, K. A.; Thompson, S. A.; Dawson, G. R.; Ferris, P.; Castro, J. L. 3-Phenyl-6-(2-pyridyl)methyloxy-1,2,4-triazolo[3,4-*a*]phthalazines and Analogues: High-affinity γ-aminobutyric acid-A benzodiazepine receptor ligands with α2-, α3-, and α5-subtype binding selectivity over α1. *J. Med. Chem.* **2004**, *47*, 1807.

16. (a) Sinkkonen, J.; Ovcharenko, V.; Zelenin, K. N.; Bezhan, I. P.; Chakchir, B. A.; Al-Assar, F.; Pihlaja, K. ^{1}H and ^{13}C NMR study of 1-hydrazino-2,3-dihydro-1*H*-pyrazolo[1,2-*a*]pyridazine-5,8-diones and −1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones and their ring-chain tautomerism. *Eur. J. Org. Chem.* **2002**, 2046–2053; (b) Jain, R. P.; Vederas, J. C. Structural variations in keto-glutamines for improved inhibition against hepatitis A virus 3C proteinase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3655–3658.
17. Ghahremanzadeh, R.; Shakibaei, G. I.; Bazgir, A. An efficient one-pot synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives. *Synth. Lett.* **2008**, 1129–1132.
18. Nabid, M. R.; Rezaei, S. J. T.; Ghahremanzadeh, R.; Bazgir, A. Ultrasound-assisted one-pot, three-component synthesis of 1H-pyrazolo[1,2-b]phthalazine-5,10-diones. *Ultrason. Sonochem.* **2010**, *17*, 159–161.
19. Ghahremanzadeh, R.; Ahadi, S.; Sayyafi, M.; Bazgir, A. Reaction of phthalhydrazide and acetylenedicarboxylates in the presence of N-heterocycles: An efficient synthesis of phthalazine derivatives. *Tetrahedron Lett.* **2008**, *49*, 4479–4482.
20. Liu, L.-P.; Lu, J.-M.; Shi, M. $PhI(OAc)_2$-mediated novel 1,3-dipolar cycloaddition of methylenecyclopropanes (MCPs), vinylidenecyclopropanes (VCPs), and methylenecyclobutane (MCB) with Phthalhydrazide. *Org. Lett.* **2007**, *9*, 1303–1306.
21. Amer, A.; Nagwa, R.; Mohamed, M.; Abdel, R. Pyrazoles derived from dihydralazine. *Heterocycles* **1987**, *26*, 1277–1283.
22. Becke, A. D. Density-functional thermochemistry, III: The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
23. Lee, C.; Yang, W.; Parr, R. G. Development of the Colle–Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B* **1988**, *37*, 785–789.
24. Arif, J. M.; Kunhi, M.; Bekhit, A. A.; Subramanian, M. P.; Hussein, K. A.; Enein, H. Y. A.; Khodairy, F. M. Evaluation of apoptosis induction by newly synthesized phthalazine derivatives in breast cancer cell lines. *Asian Pacific J. Cancer Prev.* **2006**, *7*, 249–252.
25. Ford, C. H. J.; Richardson, V. J.; Tsaltas, G. Comparison of tetrazolium colorimetric and [3H] uridine assays for in vitro chemosensitivity testing. *Pharmacology* **1989**, *24*, 295–301.
26. Shaikh, I. A.; Johnson, F.; Grollman, A. P. Structure–activity relationships among simple bicyclic analogs: Rate dependence of DNA degradation on quinone reduction potential. *J. Med. Chem.* **1986**, *29*, 1329–1340.