Synthesis and Antiproliferative Activity Evaluation of 1,3-Diarylpyrazolones as Potential Anti-lung Cancer Agents

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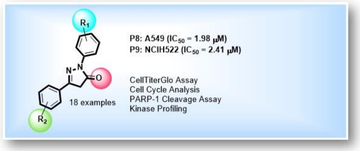
Abstract



In the U.S., lung cancer is by far the leading cause of cancer-related deaths, and approximately 85% of lung cancers are within a group of histological subtypes collectively known as non-small cell lung cancer (NSCLC). Currently available lung cancer therapies have various limitations, such as drug resistance and undesired off-target effects so, there is an unmet need for the discovery of new anticancer therapeutics that are more selective, potent and less toxic. Nitrogen heterocyclic compounds are an integral part of a huge number of natural and synthetic compounds that play important roles in the biological systems. Along those lines, a series of pyrazole heterocyclic compounds with different substitution patterns have been synthesized using microwave reaction conditions and evaluated their in vitro antiproliferative activities against two NSCLC cell lines (A549 and NCIH522). Among the tested compounds, 1,3-diarylpyrazolones with the halo substituents showed at least 10-fold higher activity (IC50~2 ⍰M) compared to positive control Celecoxib. Moreover, some pyrazolones exhibited high selectivity index with promising IC50 values (6.35 and 4.70 ⍰M) against NSCLC cell lines compared normal human lung fibroblast cell line (IC50 = 61.26 ⍰M). Further mechanistic studies revealed that the potent pyrazolones arrest the cell cycle at G2/M phase in NCIH522 cells. Additionally, western blotting in NCIH522 cells indicates the enhanced expression of the cleaved PARP-1 in a dose-dependent manner demonstrating that our compounds induce apoptosis by activating mitochondrial intrinsic pathway. Therefore, the results indicated that the 1,3-diarylpyrazolone compounds are potent and selective towards lung cancer cells and they can be promising anti-lung cancer drug candidates for further study.

Significance of Project

- Cancer remains one of the leading causes of death in the world despite immense advances in the field of basic and clinical research
- Lung cancer is the most common type of cancer with a morbidity rate of 11.6% and a mortality rate of 18.4% globally.
- Approximately 85% of lung cancers are within a group of histological subtypes collectively known as non-small cell lung cancer (NSCLC) of which lung adenocarcinoma and lung squamous cell carcinoma are the most common.
- Although the treatment with tyrosine kinase inhibitors (TKIs) has improved the outcomes for patients with NSCLC, acquired resistant to TKIs has caused treatment failure with new non-TKI therapeutics desperately needed.
- Nitrogen heterocyclic compounds such as pyrazole and pyrazolone derivates attracted huge attention in recent years due to wide-spread application as pharamaceutical agents, synthetic scaffolds in combinatorial and medicinal chemistry.
- Moreover, the broad spectrum of pharmacology properties about pyrazolone derivatives have been reported anti-microbial, anti-oxidant, anti-inflammatory, anti-tubercular, antitumor, CNS activity, antiviral, lipid-lowering, antihyperglycemic, and protein inhibitory activities

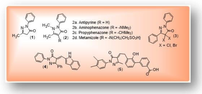


Figure 1. Structures of pyrazolone based drug molecules

Structural Features of Pyrazolones and Microwave Assisted Synthesis

Structural Features of Pyrazolones:

- Despite numerous studies reported on biological activities pyrazolone derivatives, most of the reports indicate the use of 1-aryl-3-alkylpyrazol-5-ones with various substitutions at 4-position (6, Figure 2). Relatively, a small group of compounds contain 1-aryl-3-phenyl pyrazolones with the variations on rings 'A' and 'B' but not on 'C' (7).
- Moreover, it is surprising to see that a very little focus is given to 1,3-diarylpyrazol-5-ones with different substitutions on ring B (at position 4).
- Therefore, we were interested in synthesizing the less explored class of pyrazolone derivatives (8) and investigate their antiproliferative activity with the main goal of identifying anticancer agents.

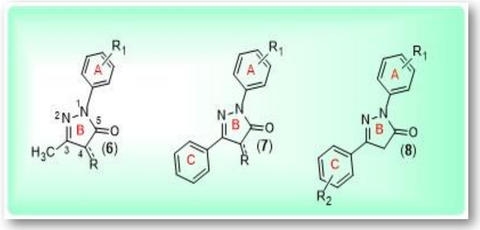


Figure 1. Structural features of pyrazolones

Microwave Assisted Synthesis:

- Compared to conventional heating procedures, microwave-assisted reactions are often much faster, cleaner, and able to produce high yields. Accordingly, we developed microwave assisted synthetic approach for the synthesis of 17 pyrazolone derivatives, which we screened against A549 and NCIH522 cell lines for their antiproliferative activities

Figure 2. Microwave assisted synthesis of pyrazolones

1-3-Diaryloyrazolones and their antiproliferative activity

Table 1. Microwave assisted synthesis of 1,3-disubstituted pyrazol-5-ones

Code	Structure	% Yield	Code	Structure	% Yield [a]
P1		86	P11		81
P2		73	P12		74
P3		88	P13		72
P4		91	P14		76
P5		87	P15		70
P6		72	P16		67
P7		78	P17		71
P8		75	P18		66
P9		66			
P10		72			

Table 2. Antiproliferative activity of the synthesized pyrazolone compounds

S. No.	IC_{50} (μM)			S. No.	IC_{50} (μM)		
	A549	NCIH522	Normal HLF*		A549	NCIH522	Normal HLF*
P0[b]	29.84 ±0.35	21.35±0.70	ND	P10 (C24)	>100	>100	ND
P1 (C16)	>200	>200	ND	**P11 (C22)**	5.98±0.84	4.88±1.73	26.06 ± 1.12
P2 (C15)	>100	>100	ND	P12 (C23)	8.6 ± 0.59	9.9 ±2.44	ND
P3 (C7)	10.35 ± 1.55	17.01 ± 2.61	ND	P13 (C20)	2.67± 0.51	3.70 ± 0.34	ND
P4 (C34)	2.61± 1.12	4.93 ± 1.13	0.72± 1.07	P14 (C21)	2.73±0.28	2.41±0.57	ND
P5 (C35)	1.98± 1.10	4.50 ± 1.16	0.99± 1.25	P15 (C37)	3.58± 0.88	10.22 ± 1.30	1.07± 1.30
P6 (C36)	39.56± 1.05	40.5 ± 2.30	26.84± 1.40	P16 (C25)	3.37 ± 0.10	2.95 ± 0.18	ND
P7 (C13)	6.35 ± 0.85	4.70 ± 1.38	61.26 ± 1.08	P17 (C27)	5.56 ±0.07	5.07±1.18	ND
P8 (C17)	9.51±3.12	17.88 ±3.36	ND	P18 (C33)	6.89 ± 1.29	3.88 ±0.41	ND
P9 (C14)	42.25 ± 2.76	51.25 ± 3.06	ND	P19 (C38)	51.09 ± 1.14	61.70 ± 1.12	45.10 ± 1.15

*Normal Human Lung Fibroblast Cells, [b]P0 = positive control (Celecoxib)

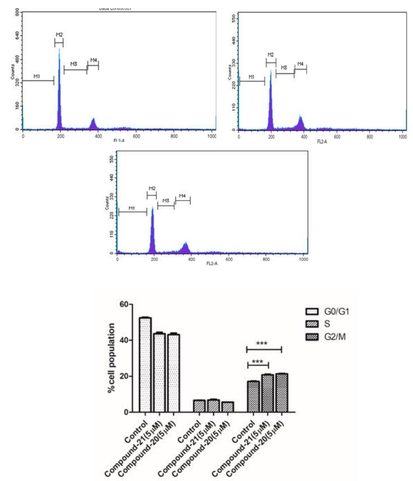
Table 3. Selective inhibition of lung cancer cells compared to skin cancer cells

	A549	NCIH522	GFP A375	SKMEL-28
P14	2.73±0.28	2.41±0.57	28.7±1.17	31.2±2.39
P16	3.37 ± 0.10	2.95 ± 0.18	31.2±1.47	27.4±2.25

Cell Cycle Analysis and PARP Cleavage Assay

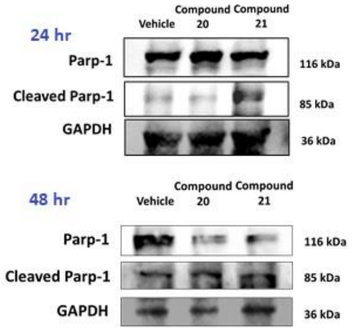
Cell Cycle Analysis:

- After testing the inhibitory effect of pyrazolones on cell proliferation, the effect of compounds was examined by flow cytometry in NCI-H522 cells.
- Treatment of NCI-H522 cells with P13 and P14 for 48hrs at 5 mM concentration resulted in more cell population in G2/M phase with a corresponding decrease of cell percentage in both G1 and S phases as shown in Figure 4. These findings were confirmed that the potent pyrazolones could influence NCI-H522 cell cycle progression at low micromolar concentrations in a dose-dependent manner



PARP Cleavage Assay:

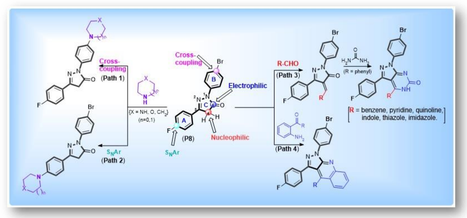
- Because of the similarity in cell cycle observations with PARP inhibitors, PARP-1 cleavage assay was performed to gain additional insights on mechanisms underlying the antiproliferative effect of P13 and P14 on NCI-H522 cells.
- We found that compounds P8 and P9 induced PARP1 fragmentation, generating poly (ADPribosyl)ated 89-kDa and 24-kDa PARP1 fragments. The result demonstrates that our compounds P13 and P14 induce apoptosis by activating mitochondrial intrinisic pathway



Conclusions and Future Directions

- We have synthesized a series of pyrazolone compounds using a microwave-assisted approach that displayed high inhibitory potential against two non-small cell lung cancer cell lines with low IC50 values (2.60μM and 2.41μM).
- Cell cycle analysis, PARP cleavage assay and kinase profiling assays were performed to gain addition insights on mechanism of action
- While this initial chemistry and pharmacology effort has produced interesting pyrazolone analogs we continue to see compounds, which are more potent and have improved ADME properties (i.e., lower IC50, improved metabolic stability, longer half-life) through structure-based design

Design of Pyrazolone Hybrid Molecules



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