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Synthesis and in vitro anticancer and antitubercular activity of diarylpyrazole ligated dihydropyrimidines possessing lipophilic carbamoyl group

Rama Krishna Yadlapalli [a], O.P. Chourasia [a], Kiranmayi Vemuri [b], Manjula Sritharan [b], Ramu Sridhar Perali [c,*]

[a] Department of Chemistry, Dr. Harisingh Gour University (A Central University), Sagar 470 003, MP, India
[b] Department of Animal Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India
[c] School of Chemistry, University of Hyderabad, Hyderabad 500 046, India

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ABSTRACT

A series of dihydropyrimidine derivatives were synthesized by utilizing Biginelli reaction and evaluated for their in vitro anticancer activity against MCF-7 human breast cancer (HBC) cell line using sulforhodamine B (SRB) assay and antitubercular activity against *Mycobacterium tuberculosis* (MTB) $H_{37}Rv$ using Microplate Alamar Blue Assay (MABA). Compounds **13p**, **13t** were exhibited 70.6% and 63.7% of HBC cell growth inhibition at 10 μM concentration. Interestingly compound **13p** was also found to be the most potent in the series against MTB $H_{37}Rv$ with MIC value of 0.125 μg/mL.

Substituted 1,3-diarylpyrazoles[1] and 4-aryl-3,4-dihydro-2(1*H*)-pyrimidone esters (DHPMs)[2] are found to be interesting molecular scaffolds for de-novo drug design towards the identification of novel non-steroidal anti-inflammatory drug (NSAID)s, antiviral and anticancer agents. Diverse analogs of 1,5-diarylpyrazoles also reported to exhibit impressive cyclooxygenase-2 (COX-2) inhibitory potency.[3] Due to the elevated levels of COX-2 enzyme in breast cancer cells, COX-2 is one of the targets to be selectively inhibited to suppress osteolytic bone metastasis as well as angiogenesis in tumor cells. In this direction several NSAIDs were synthesized and their COX-2 inhibitory activities were evaluated. As a result, celecoxib (Celebrex®) **1**, a selective COX-2 inhibitor is in the market for the treatment of both osteoarthritis and adult rheumatoid arthritis and monastrol **2**, a mitotic kenensin EG5 inhibitor[4] were identified (Fig 1). A similar kind of molecular skeleton is present in nitractin **3**, an antiviral agent. 1,3-Diarylpyrazole derivative, lonazolac **4** (Irritren®), is also in the market for the treatment of inflammatory diseases. DHPMs are also important structural motifs present in potent calcium channel blockers, antihypertensive agents,[5] adrenergic antagonists[6] and neuropeptide Y (NPY) antagonists,[7] fatty acid transporter FATP4 inhibitors.[8] Very recently DHPMs with potential anticancer activity against MCF-7 human breast cancer cells[9] was reported.

On the other hand pyrimidine derivatives were also evaluated as selective inhibitors of dihydrofolate reductase (DHFR), a key enzyme in the folate cycle,[10] from *Mycobacterium tuberculosis* (MTB).[11] Diarylpyrazole based dihydropyrimidine derivatives were found to be the most active compounds in vitro with MIC values upto 0.02 μg/mL against MTB and were more potent than isoniazid.[12] Several DHPM derivatives bearing a lipophilic group (carbamoyl moiety) were also shown to be potential antitubercular agents.[13] These compounds could act as precursors which after penetration of the compound into the cell wall of MTB would be converted to the carboxylate anions by enzymatic hydrolysis.[14]

The above observations prompted us to evaluate the bioactivity of 1,3-diarylpyrazole ligated DHPMs possessing lipophilic carbamoyl moiety. Toward the synthesis of these double pharmacophore containing molecules, we adopted a general and diversity oriented multi component reaction (MCR) involving a one pot condensation of an activated 1,3-dicarbonyl system, 1,3-diarylpyrazole carbaldehyde and urea or thio-urea.

Differentially substituted acetoacetanilide derivatives possessing electron releasing groups (ERG) and electron withdrawing groups (EWG) on the anilide were selected for the MCR reaction. These acetoacetanilides (**8a**–**8f**) were prepared by refluxing

* Corresponding author.
E-mail address: prssc@uohyd.ernet.in (R.S. Perali).

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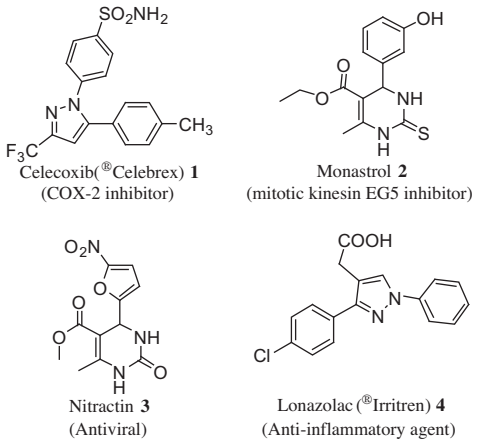
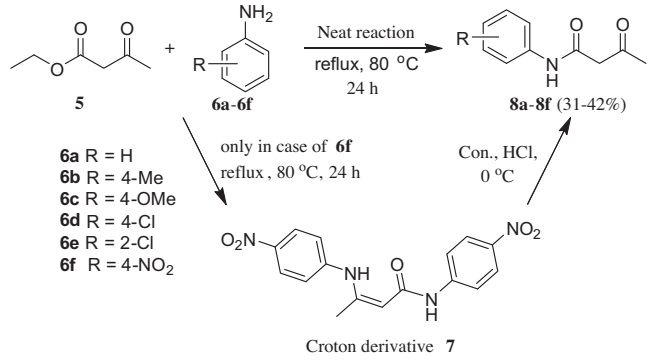
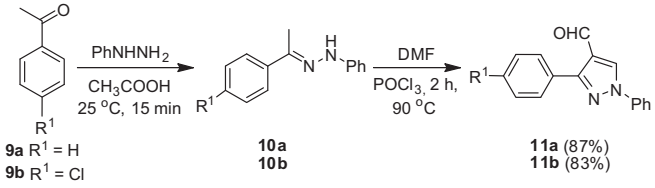


Figure 1. Dihydropyrimidine and diarylpyrazole structures with biological importance.



Scheme 1. Synthesis of acetoacetanilide derivatives.



Scheme 2. Synthesis of 1,3-diaryl-1*H*-pyrazole-4-carbaldehydes.

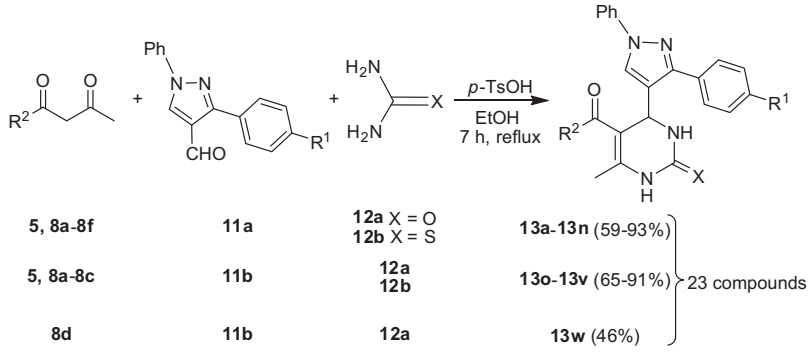
equimolar amounts of corresponding substituted anilines and ethylacetoacetate under solvent free conditions (Scheme 1).[15]

A two step protocol was adopted for the synthesis of 1,3-diarylpyrazole-4-carbaldehyde derivatives. Accordingly, reaction of acetophenones (**9a** and **9b**) with phenylhydrazine in acetic acid at 25 °C provided the corresponding hydrazones **10a** and **10b**. These hydrazones upon reaction with excess Vilsmeier reagent, with the in situ generated halomethyleneiminium salt, resulted the formation of 1,3-diarylpyrazole-4-carbaldehyde derivatives **11a** and **11b** in excellent yield (Scheme 2). The expected target dihydropyrimidine derivatives (**13a**–**13w**) were prepared by refluxing equimolar amounts of β-ketoester (ethylacetoacetate, **5**)/acetoacetanilides (**8a**–**8f**), pyrazole-carbaldehydes (**11a** and **11b**) and excess amount of urea/thiourea (1.5 equiv) with catalytic amount of *p*-toluenesulfonic acid (40 mol %) in ethanol (Scheme 3).

Interestingly better yields were obtained using thiourea compared to urea. The formation of DHPMs was confirmed by observing the characteristic peaks of the three components that were used for MCR in the product NMR. All the DHPMs showed the asymmetric CH proton in ^{1}H NMR spectrum between δ 5.30–5.80 ppm, as well as a singlet at δ 2.00–2.40 ppm equivalent to the methyl group on the dihydropyrimidine ring. Further in ^{13}C NMR spectra showed chemical shifts at δ 173.0–175.0 ppm corresponding to C═S and δ 152.0–154.0 ppm assigning to C═O on the dihydropyrimidine ring. The ^{13}C NMR peaks of carbonyl present in ester and carbamoyl group were found to be in between δ 164.0–167.0 ppm. The electron rich quaternary carbon of DHPM which is adjacent to the asymmetric center was observed in between δ 99.0–109.0 ppm as expected. All the final products were also confirmed by taking LC–MS and observing the molecular ion peak. Finally the elemental analysis results were within ±0.4% of the theoretical values assuring the purity of the final products for bioactivity studies.

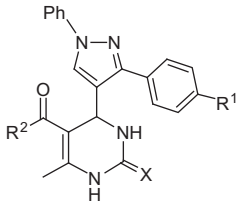
However, when R^1 is H, X is O and R^2 is either 4-$CH_3OC_6H_4NH$ or 4-ClC_6H_4NH (**13g** and **13i**) the products were obtained as diastereomeric mixtures in the ratio of 71:29 and 83:17, respectively.[16] Due to this reason these two compounds were not further evaluated for bioactivity studies. Moreover, this observation also confirms that substituent's at R^1 and on phenyl ring of the carbamoyl group play an important role in the product confirmation.

Out of 23 compounds (**13a**–**13w**) that were synthesized 21 were subjected for in vitro anticancer screening against MCF-7 human breast cancer cells using SRB assay protocol.[17] Each compound was tested at four dose levels (1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M and 1×10^{-4} M). Appropriate positive controls were run in each experiment and each experiment was repeated thrice. As shown in the Table 1 six compounds, **13b**, **13j**, **13m**, **13n**, **13p** and **13t**, were found to inhibit growth of MCF-7 cells at GI50 (concentration of compound causing 50% inhibition of cell growth) of less than 50.0 μM. Out of all, compounds **13p** (entry 16), **13t** (entry 20) exhibited excellent growth inhibition of MCF-7 HBC cell lines with 70.6% and 63.7%, respectively at 10 μM concentration.



Scheme 3. One-pot synthesis of 1,3-diarylpyrazole ligated dihydropyrimidine derivatives.

Table 1
Inhibitory effect of the synthesized compounds upon MCF-7 cells and *Mycobacterium tuberculosis* $H_{37}Rv$



13a-13w

					Breast Cancer Cell Line MCF-7			MTB $H_{37}Rv$
					% Growth inhibition		Growth inhibition	Visual MABA MICs[d]
Entry	R^1	R^2	X	Products	at 10^{-5} M (10 μM)	at 10^{-4} M (100 μM)	GI50(μM)[c]	(μg/mL)
1	H	OC_2H_5	O	**13a**	<10	86	60.8	16
2	H	OC_2H_5	S	**13b**	30.4	−12.0[b]	**42.0**	32
3	H	C_6H_5NH	O	**13c**	<10	63.2	80.6	32
4	H	C_6H_5NH	S	**13d**	<10	57.4	88.2	32
5	H	4-$CH_3C_6H_4NH$	O	**13e**	11.9	68.6	71.7	32
6	H	4-$CH_3C_6H_4NH$	S	**13f**	19.7	84.3	56.1	16
7	H	4-$CH_3OC_6H_4NH$	O	**13g**[a]	–	–	–	–
8	H	4-$CH_3OC_6H_4NH$	S	**13h**	<10	63.9	77.3	8
9	H	4-ClC_6H_4NH	O	**13i**[a]	–	–	–	–
10	H	4-ClC_6H_4NH	S	**13j**	48.6	−12.8[b]	**34.7**	8
11	H	2-ClC_6H_4NH	O	**13k**	13.9	72.2	66.6	32
12	H	2-ClC_6H_4NH	S	**13l**	16.5	68.5	68.8	16
13	H	4-$NO_2C_6H_4NH$	O	**13m**	27.3	−17.7[b]	**35.5**	16
14	H	4-$NO_2C_6H_4NH$	S	**13n**	48.4	99.3	**41.4**	32
15	Cl	OC_2H_5	O	**13o**	17.1	70.3	68.8	**0.25**
16	Cl	OC_2H_5	S	**13p**	**70.6**	**99.2**	**33.2**	**0.125**
17	Cl	C_6H_5NH	O	**13q**	19.6	71.7	65.5	**2**
18	Cl	C_6H_5NH	S	**13r**	24.2	74.7	61.5	32
19	Cl	4-$CH_3C_6H_4NH$	O	**13s**	13.6	70.4	69.7	32
20	Cl	4-$CH_3C_6H_4NH$	S	**13t**	**63.7**	**84.9**	**43.5**	**2**
21	Cl	4-$CH_3OC_6H_4NH$	O	**13u**	<10	72.1	69.7	**1**
22	Cl	4-$CH_3OC_6H_4NH$	S	**13v**	<10	72.0	70.0	16
23	Cl	4-ClC_6H_4NH	O	**13w**	<10	88.7	56.9	32

GI50 = Concentration of compound causing 50% inhibition of cell growth. The bold fonts indicate the best activity values obtained in bioactivity studies, out of all the compounds that were screened.

[a] Obtained as a diastereomeric mixture so that the activity tests were not conducted.

[b] % Control growth, negative numbers indicate the cell kill.

[c] Each compound is tested at four dose levels 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, and each experiment is repeated thrice.

[d] The experiment was done in duplicate each time and was done twice to confirm the MIC values and confirmed in the BacT/ALERT®MP Mycobacteria detection system (bioMérieux, France).

Compound **13p** showed maximum inhibition of cell growth with GI50 of 33.2 μM. In addition **13p** also exhibited good dose–response (Fig. 2). The above structure–activity relationship (SAR) studies revealed that compounds containing X = S were more potent than the X = O counter parts. Based on this observation we may conclude that presence of thio-urea functional group in DHPMs enhances the anticancer activity of these type of scaffolds.

Due to the presence of 1,3-diarylpyrazole along with lipophilic carbamoyl moiety all the 21 compounds were also tested against MTB $H_{37}Rv$. The minimum inhibitory concentration (MIC) values of compounds (**13a**–**13w**) were obtained by the Microplate Alamar Blue Assay[18] (MABA) after 5 days of incubation at 37 °C (Table 1, column 9). The experiment was done in duplicate each time and was repeated twice to check the reproducibility of the MIC values and also confirmed in the BacT/ALERT®MP Mycobacteria detection system (bioMérieux, France). More interestingly, compounds **13p** and **13o**, with a MIC value of 0.125 and 0.25 μg/mL, were found to be the most potent in the series. Compounds **13q**, **13t** and **13u** were also expressed good MIC values of 2.0, 2.0 and 1.0 μg/mL, respectively. These compounds could be derivatized further towards the discovery of more potent antimicrobial agents. The above SAR studies revealed that compounds with chlorine (Cl) at R^1 in the series, were more potent (Compounds **13o**, **13p**, **13q**, **13t** and **13u**) when compared to compounds in which R^1 = H. These results also suggest that replacing the alkyl ester moiety (R^2) with lipophilic carbamoyl does influence the GI50 values against MCF-7 breast cancer cells as well as MIC values against MTB $H_{37}Rv$.

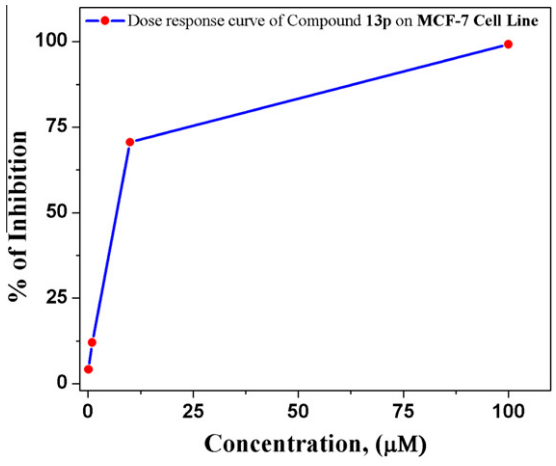


Figure 2. Dose response curve for in vitro anticancer activity of **13p** at 0.1 μM 4.1%, 1 μM 12%, 10 μM 70.6% and 100 μM 99.2%.

In conclusion, a Biginelli reaction was implemented towards the synthesis of diarylpyrazole with DHPM possessing lipophilic carbamoyl groups. These novel scaffolds showed moderate anticancer activity against MCF-7 breast cancer cell lines as well as good to excellent antitubercular activity against MTB $H_{37}Rv$. Out of all, compound **13p** was found to be the most effective and potent molecule against both anticancer and antitubercular activity. The

biological potency of this series of molecules renders them attractive leads for further exploration. With respect to the potential bioactivity of polyfunctionalized dihydropyrimidines, further evaluation of the above analogs is under progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.101.

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