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Synthesis and Biological Evaluation of *vicinal*-Diaryl Pyrazole Ethyl Carboxylate Analogs as Antiproliferative Agent against Pancreatic Cancer

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vicinal Diaryl scaffold possessing various heterocycles displayed versatile pharmacological activities ranging from antibacterial to antiviral. Herein, the synthesis of novel *vicinal* diaryl pyrazole ethyl carboxylate analogs as central ring and evaluated for their antiproliferative activity against pancreatic cancer line, PANC-1. Among the synthesized 27 compounds, six compounds displayed the IC_{50} value for antiproliferative activity in single digit micromolar. The cytotoxicity results of the synthesized compounds especially compound **25** (IC_{50} = 4.8 μM) confirms that these analogs may require further investigation.

Keywords: *vicinal* Diaryl, Pyrazole, Cytotoxicity, Pancreatic cancer lines.

INTRODUCTION

The importance of heterocyclic compounds and its derivatives in the new drug discovery is attracted owing to their superior pharmacological activity. It is well documented that the heterocyclic compounds serves as precursor for various pharmaceuticals, organaocatalysts, veterinary medicines, polymers, dietary supplements, agrochemicals, *etc*. Over half of the medications approved by the US Food and Drug Administration (FDA) contain at least one heterocycle moiety [1-3]. 1,2-Azoles such as pyrazole, isoxazole and isothiazoles are the important members in the heterocyclic chemistry. Among the 1,2-azoles, pyrazole and its analogs occupied a prominent position in the medicinal chemistry due to its versatility. Over the past few decades, USFDA has approved more than 40 pyrazole containing molecules for various therapeutic categories [4]. In addition, pyrazole containing drugs may possess enhanced pharmacokinetic properties owing to its adjoining positions of two nitrogen atoms in the ring and act as hydrogen bond donor and hydrogen bond acceptor [5]. Recently, Ei-Gamal *et al*. [6] reported that pyrazole and its derivatives are capable to target various cancerous cells. Fig. 1 represents a familiar pyrazole compounds (**1-4**) as antiproliferative agents [7-10].

One of our main research interests is to synthesize the library of *vicinal* diaryl compounds hybrid with five/six/seven-membered heterocycles and investigate for diverse pharmacological action. Previously, we reported *vicinal* diaryl diazepine for antiplatelet and antileukemic activities [11]. The diaryl diazepine **6** showed superior antiplatelet activity than aspirin. The importance of *vicinal* diaryl system in various therapeutic categories including combretastatin A-4 (CA-4, **7**) is well-documentated (Fig. 2) [12]. Resveratrol (**8**) is a natural polyphenolic compound and possess similar structural features of 1,2 *vicinal* diaryl as observed in CA-4. It exhibits potent antiproliferative activity against human pancreatic and breast cancer. However, this naturally occurring resveratrol has limited activity in certain model, which may be due to the *cis-trans* isomerization. The synthetic *cis*-analog (**9**) of resveratrol showed excellent antiproliferative active against PANC-1 cell lines. Recently, Grau *et al*. [13] synthesized indolic derivative (**10**), a cyclic analog of resveratrol and evaluted the *in vitro* cytotoxic activity, which is found to be eight times higher than resveratrol against HT-29 cancer cells. Compound **10** displayed better cytotoxicity profile than *trans*-resveratrol (**8**). These results prompted us to replace the olefinic bond with pyrazole heterocycle and lead

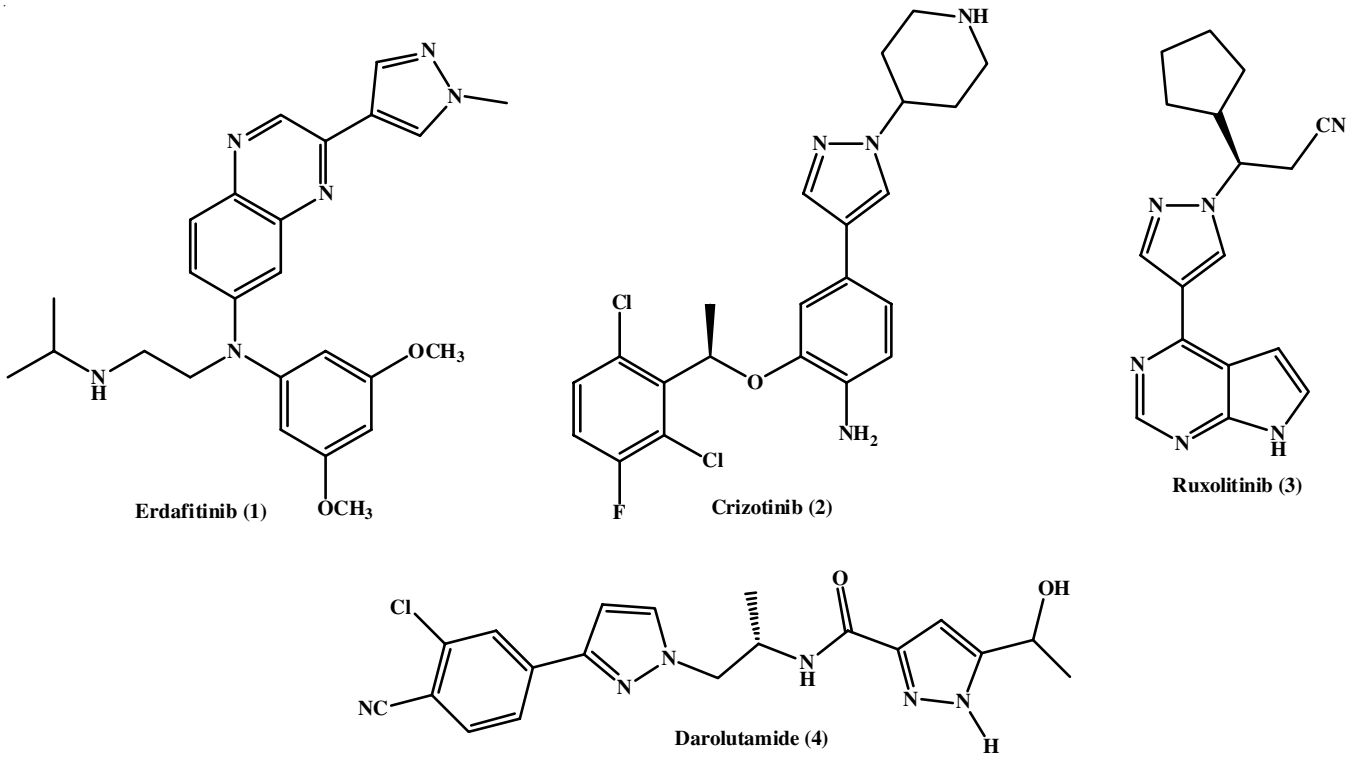


Fig. 1. FDA approved antiproliferative agent containing pyrazole moiety

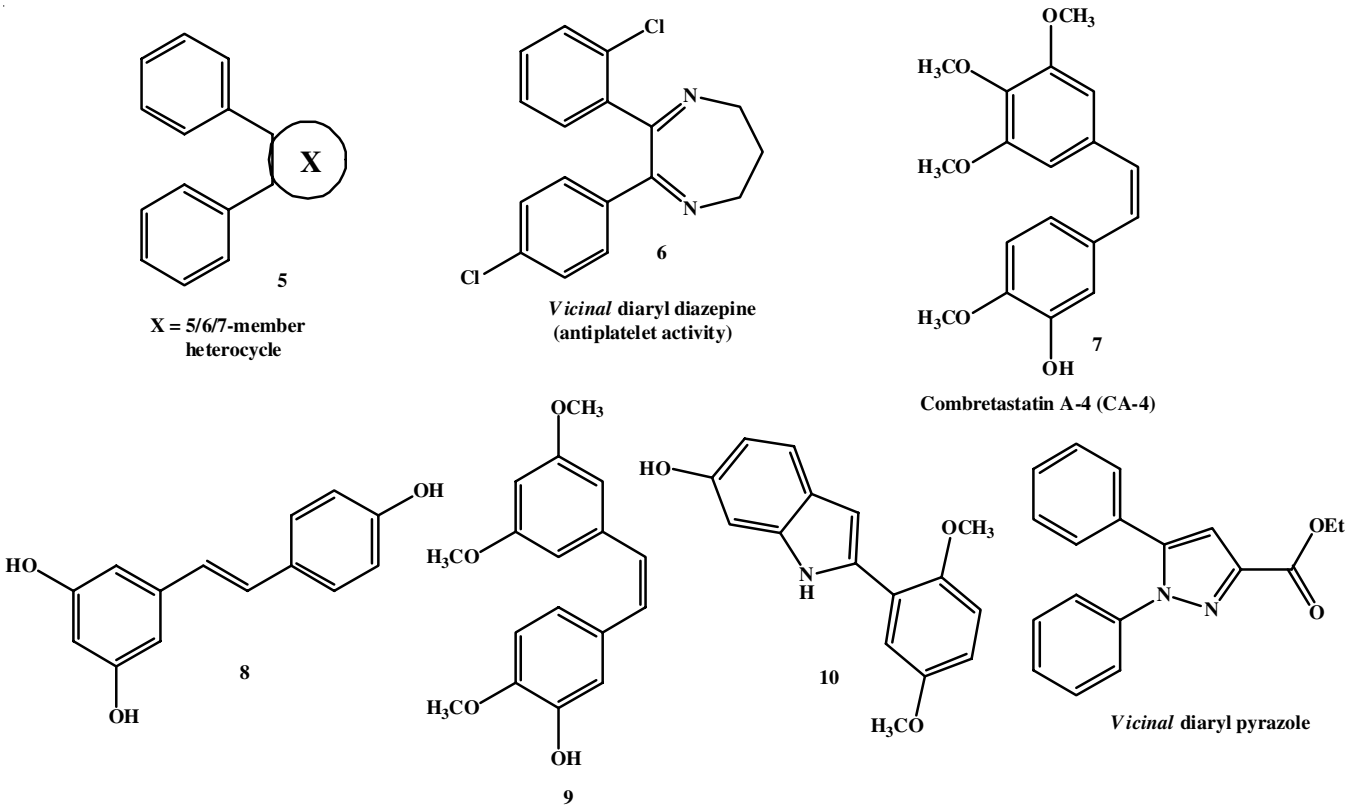


Fig. 2. *Vicinal* diaryl heterocyclic system

to formation of general structure **5**. The improved biological activity was expected on replacement of olefinic bond with the rigid heterocycle thereby maintain both the phenyl rings geometry are *cis* to each other. In this work, we aimed to synthesize *vicinal* diaryl pyrazole as antiproli-ferative agent and screened against pancreas cell line, PANC-1.

EXPERIMENTAL

All chemicals and solvents were purchased from SD Fine Chemicals (India), Spectrochem (India) and Loba Chemicals (India) and used without any further purification. The progress of the reactions were monitored by thin-layer chromatography (TLC). TLC plates were visualized by illuminating with UV light (254 nm) or exposure to iodine vapours. ^{1}H NMR was recorded using Bruker AVANCE 500 MHz spectrometer. All NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using tetramethylsilane (TMS) as internal standard and the chemical shifts were expressed in ppm. Mass spectra were recorded on AB Sciex API 4000 LC-MS/MS instrument and IR spectra (KBr pellet) were recorded by on Agilent Cary 630 FTIR instrument.

General procedure for synthesis of 2,4-diketo esters (12a-i): An equimolar mixture of diethyl oxalate (42.87 mmol) and acetophenone derivatives (42.87 mmol) in methanol (20 mL) was added dropwise to sodium methoxide in MeOH (2.6 mL of 25% w/v, 12.0 mmol) and the reaction was allowed to proceed at reflux for 2 h. After cooling to 25 ºC, the reaction mixture was poured into water (40 mL), acidified with HCl (1 mL of 37% w/v) and extracted with $CHCl_3$ (3 × 30 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$, filtered and finally the solvent was removed under vacuum to afford the respective diketo esters **12a-i**. Yield and IR spectral data for **12a-i** are given in Table-1.

TABLE-1
YIELD AND IR SPECTRAL DATA FOR β-DIKETO ESTERS (**12a-i**)

Compound	R_1	Yield (%)	IR (cm^{-1})
12a	H	78	1711, 1599
12b	4-Cl	82	1700, 1588
12c	4-Br	68	1696, 1618
12d	3-Br	77	1734, 1611
12e	3,4-$(OCH_3)_2$	89	1710, 1622
12f	2-OH	65	1741, 1618
12g	2,4-Cl_2	90	1730, 1629
12h	4-OCH_3	70	1708, 1625
12i	4-CH_3	73	1700, 1603

General procedure for synthesis of *vicinal* diarylpyrazole analogs (14-40): The equimolar mixture of 2,4-diketo esters (**12a-i**) and phenylhydrazine derivatives (**13a-c**) were dissolved in ethanol (30 mL). The mixture was allowed to reflux for 6 h. The reaction was monitored throughout by TLC and the solvent was evaporated under vacuum after completion. The residue was extracted with ethyl acetate (3 × 30 mL) and then washed with water and brine solution. The organic solvent was removed under vacuum and the crude solid was collected, filtered and dried. The crude solid was then recrystallized from a suitable solvent to afford the desired compounds **14-40** (**Scheme-I**).

Ethyl 1,5-diphenyl-1*H*-pyrazole-3-carboxylate (14): Yield: 85%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1700 (C=O; ester), 1599, 1253, 1197, 922; ^{1}H NMR (DMSO-d_6) δ ppm: 0.99-1.02 (3H, t, *J* = 8 Hz), 4.28-4.34 (CH_2, q, *J* = 8 Hz), 7.03 (1H, s, pyrazole CH), 7.19-7.23 (2H, m, ArH), 7.26-7.28 (2H, m, ArH), 7.30-7.33 (3H, m, ArH); 7.40-7.42 (3H, m, ArH); MS: *m/z* 293.7 (M+1).

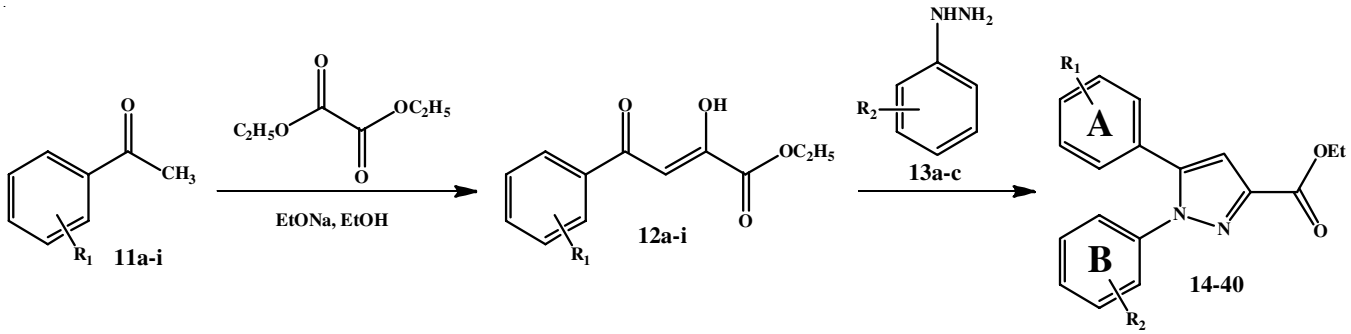
Ethyl 1-(2,4-dichlorophenyl)-5-phenyl-1*H*-pyrazole-3-carboxylate (15): Yield: 80%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1722 (C=O; ester), 1477, 1391, 1235, 1134, 769; ^{1}H NMR ($CDCl_3$) δ ppm: 1.39-1.43 (3H, t, *J* = 8 Hz), 4.41-4.45 (2H, q, *J* = 8 Hz), 7.06 (1H, s, pyrazole CH*)*, 7.16-7.18 (2H, m, ArH), 7.29-7.34 (4H, m, ArH); 7.41-7.43 (2H, m, ArH); MS: *m/z* 361.8 (M+).

Ethyl 1-(4-fluorophenyl)-5-phenyl-1*H*-pyrazole-3-carboxylate (16): Yield: 87%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1727 (C=O; ester), 1574, 1482, 1397, 1225, 1126, 772; ^{1}H NMR ($CDCl_3$) δ ppm: 1.25-1.29 (3H, t, *J* = 8 Hz), 4.26-4.31 (2H, q, *J* = 8 Hz), 7.09 (1H, s, pyrazole CH), 7.20-7.25 (3H, m, ArH), 7.26-7.28 (3H, m, ArH), 7.29-7.34 (3H, m, ArH); MS: *m/z* 311.8 (M+1).

Ethyl 5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (17): Yield: 85%; orange solid; IR (KBr, ν_{max}, cm^{-1}): 1710 (C=O; ester), 1480, 1011, 829; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.19-1.22 (3H, t, *J* = 8 Hz), 4.18-4.22 (2H, q, *J* = 8Hz), 7.05 (1H, s, pyrazole CH), 7.38-7.42 (3H, m, ArH), 7.48-7.51 (2H, m, ArH), 7.56-7.58 (2H, d, ArH), 7.86-7.88 (2H, d, ArH); MS: *m/z* 327.7 (M+1).

Ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (18): Yield: 85%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1726 (C=O; ester), 1484, 1235, 1019, 836; ^{1}H NMR (DMSO-d_6) δ ppm: 1.25-1.29 (3H, t, *J* = 8 Hz), 4.26-4.29 (2H, q, *J* =8 Hz), 7.22-7.24 (3H, m, ArH), 7.39-7.41 (2H, m, ArH), 7.60-7.63 (1H, m, ArH), 7.75-7.77 (1H, d, ArH), 7.83-7.84 (1H, d, ArH); MS: *m/z* 418.9 (M+1).

Ethyl 5-(4-chlorophenyl)-1-(4-fluorophenyl)-1*H*-pyrazole-3-carboxylate (19): Yield: 88%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1711 (C=O; ester), 1514, 1227, 1097, 829; ^{1}H NMR ($CDCl_3$) δ ppm: 1.39-1.42 (3H, t, *J* =8 Hz), 4.41-



Scheme-I: Synthesis of 1,5-diarylpyrazole analogs

4.46 (2H, q, *J* = 8 Hz), 7.01 (1H, pyrazole CH), 7.05-7.07 (2H, d, ArH), 7.11-7.13 (2H, d, ArH); 7.27-7.29 (2H, d, ArH), 7.30-7.32 (2H, d, ArH); MS: *m/z* 345.8 (M+1).

Ethyl 5-(4-bromophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (20): Yield: 81%; light brown; IR (KBr, ν_{max}, cm^{-1}): 1718 (C=O; ester), 1599, 1473, 1354, 1249; ^{1}H NMR ($CDCl_3$) δ ppm: 1.37-1.42 (3H, t, *J* = 8 Hz), 4.41-4.46 (2H, q, *J* = 8 Hz), 7.03 (1H, pyrazole CH), 7.43-7.45 (1H, m, ArH), 7.58-7.62 (4H, m, ArH), 7.64-7.66 (2H, d, ArH), 7.76-7.78 (2H, d, ArH); MS: *m/z* 371.04 (M+).

Ethyl 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (21): Yield: 81%; light brown; IR (KBr, ν_{max}, cm^{-1}): 1726 (C=O; ester), 1484, 1231, 1015, 871; ^{1}H NMR (DMSO-d_6) δ ppm: 1.23-1.27 (3H, t, *J* = 8 Hz), 4.22-4.26 (2H, q, *J* =8 Hz), 7.02 (1H, pyrazole CH), 7.21-7.24 (2H, m, ArH), 7.36-7.38 (2H, m, ArH), 7.60-7.63 (1H, m, ArH), 7.75-7.77 (2H, m, ArH), MS: *m/z* 441.8 (M+1).

Ethyl 5-(4-bromophenyl)-1-(4-fluorophenyl)-1*H*-pyrazole-3-carboxylate (22): Yield: 84%; orange solid; IR (KBr, ν_{max}, cm^{-1}): 1689 (C=O; ester), 1514, 1242, 1011, 840; ^{1}H NMR ($CDCl_3$) δ ppm: 1.39-1.42 (3H, t, *J* =8 Hz), 4.40-4.44 (2H, q, *J* = 8 Hz), 7.00 (1H, pyrazole CH), 7.22-7.24 (2H, d, ArH), 7.58-7.60 (2H, d, ArH); 7.62-7.64 (2H, d, ArH), 7.78-7.80 (2H, d, ArH); MS: *m/z* 389.9 (M+1).

Ethyl 5-(3-bromophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (23): Yield: 80%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1730 (C=O; ester), 1566, 1482, 1246, 775; ^{1}H NMR ($CDCl_3$) δ ppm: 1.31-1.36 (3H, t, *J* = 8 Hz), 4.40-4.45 (2H, q, *J* = 8 Hz), 7.01 (1H, pyrazole CH), 7.40-7.41 (2H, m, ArH), 7.58-7.62 (2H, m, ArH), 7.63-7.66 (3H, d, ArH), 7.76-7.78 (2H, d, ArH); MS: *m/z* 371.64 (M+).

Ethyl 5-(3-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (24): Yield: 89%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1722 (C=O; ester), 1484, 1235, 1127, 817; ^{1}H NMR (DMSO-d_6) δ ppm: 1.18-1.22 (3H, t, *J* = 8 Hz), 4.20-4.23 (2H, q, *J* = 8 Hz), 7.05 (1H, pyrazole CH), 7.32-7.34 (2H, m, ArH), 7.38-7.41 (2H, m, ArH), 7.53-7.55 (1H, m, ArH), 7.62-7.64 (1H, d, ArH), 7.69-7.71 (1H, d, ArH); MS: *m/z* 440.96 (M+1).

Ethyl 5-(3-bromophenyl)-1-(4-fluorophenyl)-1*H*-pyrazole-3-carboxylate (25): Yield, 92%; light yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1715 (C=O; ester), 1518, 1235, 1119, 847; ^{1}H NMR ($CDCl_3$) δ ppm: 1.39-1.42 (3H, t, *J* = 8 Hz), 4.43-4.46 (2H, q, *J* =8 Hz), 7.03 (1H, pyrazole CH), 7.14-7.18 (2H, m, ArH), 7.30-7.31 (1H, m, ArH), 7.45-7.47 (1H, m, ArH), 7.74-7.76 (2H, d, ArH), 8.01-8.02 (2H, d, ArH); MS: *m/z* 389 (M+).

Ethyl 5-(3,4-dimethoxyphenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (26): Yield: 86%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1722 (C=O; ester), 1570, 1462, 1149, 1026, 765; ^{1}H NMR (DMSO-d_6) δ ppm: 1.39-1.42 (3H, t, *J* = 8 Hz), 4.25-4.29 (2H, q, *J* =8 Hz), 3.51 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 6.72-6.75 (1H, d, ArH), 6.85-6.88 (1H, d, ArH), 7.07 (1H, pyrazole CH), 7.23-7.25 (1H, d, ArH), 7.29-7.33 (2H, m, ArH), 7.42-7.44 (3H, m, ArH); MS: *m/z* 353 (M+1).

Ethyl 1-(2,4-dichlorophenyl)-5-(3,4-dimethoxyphenyl)-1*H*-pyrazole-3-carboxylate (27): Yield: 87%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1710 (C=O; ester), 1488, 1235, 1027, 814; ^{1}H NMR ($CDCl_3$) δ ppm: 1.28-1.31 (3H, t, *J* = 8 Hz), 3.83 (3H, s, OCH_3), 3.9 (3H, s, OCH_3), 4.36-4.40 (2H, q, *J* =8 Hz), 6.70-6.72 (1H, d, ArH), 6.82-6.84 (1H, d, ArH), 7.04 (1H, pyrazole CH), 7.31-7.32 (1H, m, ArH), 7.38-7.41 (1H, m, ArH), 7.53-7.55 (1H, m, ArH), 7.62-7.64 (1H, d, ArH); MS: *m/z* 422.4 (M+1).

Ethyl 5-(3,4-dimethoxyphenyl)-1-(4-fluorophenyl)-1*H*-pyrazole-3-carboxylate (28): Yield: 83%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1707 (C=O; ester), 1514, 1439, 1216, 1130, 814; ^{1}H NMR ($CDCl_3$) δ ppm: 1.39-1.42 (3H, t, *J* = 8 Hz), 3.86 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.36-4.40 (2H, q, *J* =8 Hz), 6.63 (1H, d, ArH), 6.77-6.80 (2H, d, ArH), 6.98 (1H, pyrazole CH), 7.04-7.06 (2H, d, ArH), 7.31-7.33 (2H, d, ArH); MS: *m/z* 371.13 (M+1).

Ethyl 5-(2-hydroxyphenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (29): Yield: 88%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1741 (C=O; ester), 1588, 1465, 1231, 1097, 858; ^{1}H NMR (DMSO-d_6) δ ppm: 1.21-1.23 (3H, t, *J* = 8 Hz), 4.23-4.26 (2H, q, *J* = 8 Hz), 6.87 (1H, pyrazole CH), 7.48-7.52 (2H, m, ArH), 7.69-7.71 (2H, m, ArH), 7.82-7.84 (2H, m, ArH), 8.00-8.03 (2H, m, ArH); MS: *m/z* 309.24 (M+1).

Ethyl 1-(2,4-dichlorophenyl)-5-(2-hydroxyphenyl)-1*H*-pyrazole-3-carboxylate (30): Yield: 88%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1730 (C=O; ester), 1599, 1467, 1272, 1100, 817; ^{1}H NMR (DMSO-d_6) δ ppm: 1.27-1.29 (3H, t, *J* = 8 Hz), 4.16-4.19 (2H, q, *J* = 8 Hz), 6.92 (1H, pyrazole CH), 7.46-7.48 (2H, m, ArH), 7.52-7.54 (1H, m, ArH), 7.65-7.67 (2H, m, ArH), 7.78-7.80 (2H, m, ArH); MS: *m/z* 377.8 (M+).

Ethyl 1-(4-fluorophenyl)-5-(2-hydroxyphenyl)-1*H*-pyrazole-3-carboxylate (31): Yield: 83%; orange solid; IR (KBr, ν_{max}, cm^{-1}): 1741 (C=O; ester), 1592, 1465, 1231, 1100, 862; ^{1}H NMR (DMSO-d_6) δ ppm: 1.27-1.29 (3H, t, *J* = 8 Hz), 4.16-4.19 (2H, q, *J* = 8 Hz), 7.01 (1H, pyrazole CH), 7.05-7.08 (3H, m, ArH), 7.24-7.26 (2H, d, ArH), 7.52-7.54 (1H, m, ArH), 7.65-7.67 (2H, d, ArH); MS: *m/z* 327.5 (M+1).

Ethyl 5-(2,4-dichlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (32): Yield: 85%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1732 (C=O; ester), 1454, 1256, 1125, 789; ^{1}H NMR ($CDCl_3$) δ ppm: 1.29-1.32 (3H, t, *J* = 8 Hz), 4.38-4.42 (2H, q, *J* = 8 Hz), 7.03 (1H, s, pyrazole CH*)*, 7.14-7.16 (2H, m, ArH), 7.30-7.35 (4H, m, ArH); 7.41-7.43 (2H, m, ArH); MS: *m/z* 361.78 (M+).

Ethyl 1,5-*bis*(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (33): Yield: 89%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1730 (C=O; ester), 1458, 1235, 1127, 806; ^{1}H NMR (DMSO-d_6) δ ppm: 1.25-1.29 (3H, t, *J* = 8 Hz), 4.27-4.30 (2H, q, *J* = 8 Hz), 7.13 (1H, pyrazole CH), 7.31-7.33 (1H, m, ArH), 7.40-7.42 (1H, d, ArH), 7.53-7.56 (1H, dd, ArH), 7.64-7.68 (1H, d, ArH), 7.70-7.71 (1H, d, ArH) 7.77-7.78 (1H, m, ArH); MS: *m/z* 430.96 (M+1).

Ethyl 5-(2,4-dichlorophenyl)-1-(4-fluorophenyl)-1*H*-pyrazole-3-carboxylate (34): Yield: 84%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1734 (C=O; ester), 1521, 1439, 1127, 769; ^{1}H NMR (DMSO-d_6) δ ppm: 1.16-1.19 (3H, t, *J* = 8 Hz), 4.18-4.21 (2H, q, *J* = 8 Hz), 7.1 (1H, pyrazole CH), 7.32-7.34 (1d, m, ArH), 7.48-7.51 (1H, m, ArH), 7.59-7.62 (1H, m, ArH), 7.74-7.76 (2H, d, ArH) 7.84-7.86 (2H, m, ArH); MS: *m/z* 380.41 (M+1).

Ethyl 5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (35): Yield, 90%; white solid; IR (KBr, ν_{max}, cm^{-1}):

1695 (C=O; ester), 1521, 1436, 1242, 1179, 1030, 773; ^{1}H NMR (DMSO-d_6) δ ppm: 1.23-1.25 (3H, t, *J* = 8 Hz), 3.74 (3H, s, OCH_3), 4.24-4.27 (2H, q, *J* = 8 Hz), 6.85-6.87 (2H, d, ArH), 6.93 (1H, pyrazole CH), 7.11-7.13 (2H, d, ArH), 7.26-7.28 (2H, m, ArH), 7.40-7.41 (3H, m, ArH); MS: *m/z* 333.51 (M+1).

Ethyl 1-(2,4-dichlorophenyl)-5-(4-methoxyphenyl)-1*H*-pyrazole-3-carboxylate (36): Yield, 92%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1730 (C=O; ester), 1518, 1439, 1261, 1182, 1022, 836; ^{1}H NMR (DMSO-d_6) δ ppm: 1.24-1.26 (3H, t, *J* = 8 Hz), 3.81 (3H, s, OCH_3), 4.32-4.26 (2H, q, *J* = 8 Hz), 6.81-6.83 (2H, d, ArH), 6.96 (1H, pyrazole CH), 7.15-7.17 (2H, d, ArH), 7.30-7.32 (1H, m, ArH), 7.58-7.60 (1H, m, ArH), 7.70-7.71 (1H, m, ArH); MS: *m/z* 392.7 (M+1).

Ethyl 1-(4-fluorophenyl)-5-(4-methoxyphenyl)-1*H*-pyrazole-3-carboxylate (37): Yield: 81%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1700 (C=O; ester), 1514, 1227, 1192, 1033 840; ^{1}H NMR (DMSO-d_6) δ ppm: 1.29-1.31 (3H, t, *J* = 8 Hz), 3.83 (3H, s, OCH_3), 4.30-4.33 (2H, q, *J* = 8 Hz), 6.98-7.00 (2H, d, ArH), 7.02 (1H, pyrazole CH), 7.23-7.25 (2H, d, ArH), 7.42-7.44 (2H, d, ArH), 7.54-7.56 (2H, d, ArH); MS: *m/z* 341.23 (M+1).

Ethyl 1-phenyl-5-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (38): Yield: 82%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1681 (C=O; ester), 1419, 1246, 1197, 1007, 769; ^{1}H NMR (DMSO-d_6) δ ppm: 1.15-1.17 (3H, t, *J* = 8 Hz), 2.24 (3H, s, CH_3), 4.28-4.31 (2H, q, *J* = 8 Hz), 6.97 (1H, pyrazole CH), 7.07-7.13 (3H, m, ArH), 7.25-7.28 (2H, d, ArH), 7.39-7.43 (4H, m, ArH); MS: *m/z* 307.14 (M+1).

Ethyl 1-(2,4-dichlorophenyl)-5-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (39): Yield: 89%; white solid; IR (KBr, ν_{max}, cm^{-1}): 1730 (C=O; ester), 1480, 1231, 1115, 1022, 821; ^{1}H NMR ($CDCl_3$) δ ppm: 1.39-1.42 (3H, t, *J* = 8 Hz), 2.31 (3H, s, CH_3), 4.41-4.46 (2H, q, *J* = 8 Hz), 7.02 (1H, pyrazole CH), 7.04-7.09 (4H, m, ArH), 7.30-7.33 (1H, dd, ArH), 7.40-7.42 (2H, d, ArH); MS: *m/z* 375.8 (M+).

Ethyl 1-(4-fluorophenyl)-5-(*p*-tolyl)-1*H*-pyrazole-3-carboxylate (40): Yield: 98%; yellow solid; IR (KBr, ν_{max}, cm^{-1}): 1715 (C=O; ester), 1521, 1223, 1104, 844; ^{1}H NMR ($CDCl_3$) δ ppm: 1.35-1.39 (3H, t, *J* = 8 Hz), 2.38 (3H, s, CH_3), 4.28-4.32 (2H, q, *J* = 8 Hz), 7.03 (1H, pyrazole CH), 7.26-7.30 (2H, d, ArH), 7.32-7.34 (2H, d, ArH), 7.54-7.56 (2H, d, ArH), 7.62-7.64 (2H, d, ArH); MS: *m/z* 325.8 (M+1).

Antiproliferative activity: The antiproliferative effect of 1,5-diarylpyrazole analogs **(14-40)** against pancreatic cancer cell line, PANC-1, was determined by colorimetric assay using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [14]. The cells were trypsinized and 4×10^3 cells were seeded in 100 µL medium in each well of a 96-well plate (Cole Parmar). The cells were incubated at 37 ºC in a CO_2 incubator for 24 h. After 24 h, the cells were treated with increasing concentrations of 1, 10 and 100 µM of *vicinal* diaryl-pyrazole analogs. After 72 h, 20 µL of MTT (Sigma-Aldrich, USA) was added to each well and incubated at 37 ºC in a CO_2 incubator for 2 h. The formazan crystals formed were dissolved in 100 µL of stock solution, dimethyl sulphoxide (DMSO) (Qualigens). The absorbance was measured at 570 nm using a Multiskan GO microplate spectrophotometer (Thermo Scientific, USA). Percentage survival curves were plotted to calculate the half-maximal inhibitory concentrations (IC_{50}).

RESULTS AND DISCUSSION

Title compounds, *vic.*-diaryl analog containing pyrazole was synthesized in two steps as outlined in **Scheme-I**. Initially, Claisen condensation of corresponding acetophenone derivatives (**11a-i**) with diethyloxalate in presence of sodium methoxide in methanol to obtain 2,4-diketo ester (**12a-i**) [15]. The IR spectrum of 2,4-diketo esters displayed prominent intense peaks at 1618-1588 and 1740-1710 cm^{-1} for carbonyl stretching of β-diketone and ester, respectively. The yield of 2,4-diketo esters were obtained in the range of 65-90%. Subsequently, 2,4-diketo esters (**12a-i**) reacted with substituted phenyl-hydrazine derivatives (**13a-c**) furnished corresponding ethyl 1,5-diphenyl-1*H*-pyrazole-3-carboxylate analogs (**14-40**) [16]. The purification was carried out by recrystallization and yields in the range of 82-90%. The IR spectrum of cyclized compound, 1,5-diaryl pyrazole, confirmed the disappearance of carbonyl stretching of ketone of 2,4-diketo esters. The ^{1}H NMR displays quartet and triplet for CH_2 and CH_3 protons of the ester. The CH proton of pyrazole at 4th position appears as a singlet at δ 7.07 to 7.1 ppm. All the halogen compounds displayed a prominent M+2 peak in the MS spectrum.

Antiproliferative activity: The antiproliferative activity of *vicinal* diaryl pyrazole compounds was assessed against PANC-1 pancreatic cancer cell line using standard MTT assay [14,17]. The IC_{50} results are displayed in Table-2. Among 27

TABLE-2
CYTOTOXIC DATA FOR 1,5-DIARYLPYRAZOLE AGAINST PANC-1 PANCREATIC CANCER CELL LINE

Compound	R_1	R_2	IC_{50} (µm)
14	H	H	> 100
15	H	2,4-Cl_2	9.15 ± 1.1
16	H	4-F	57.15 ± 3.5
17	4-Cl	H	42.86 ± 8.2
18	4-Cl	2,4-Cl_2	52.86 ± 4.0
19	4-Cl	4-F	7.63 ± 2.1
20	4-Br	H	68.94 ± 6.5
21	4-Br	2,4-Cl_2	52.77 ± 2.0
22	4-Br	4-F	57.18 ± 4.5
23	3-Br	H	72.96 ± 6.8
24	3-Br	2,4-Cl_2	98.21 ± 4.5
25	3-Br	4-F	4.8 ± 1.0
26	3,4-$(OCH_3)_2$	H	59.1 ± 6.6
27	3,4-$(OCH_3)_2$	2,4-Cl_2	69.17 ± 6.1
28	3,4-$(OCH_3)_2$	4-F	> 100
29	2-OH	H	> 100
30	2-OH	2,4-Cl_2	98.66 ± 8.0
31	2-OH	4-F	> 100
32	2,4-Cl_2	H	9.81 ± 1.3
33	2,4-Cl_2	2,4-Cl_2	8.91 ± 0.9
34	2,4-Cl_2	4-F	7.4 ± 1.53
35	4-OCH_3	H	32.32 ± 2.06
36	4-OCH_3	2,4-Cl_2	59.51 ± 2.0
37	4-OCH_3	4-F	56.5 ± 3
38	4-CH_3	H	39.22 ± 3.07
39	4-CH_3	2,4-Cl_2	59.2 ± 5.13
40	4-CH_3	4-F	95.91 ± 8.03
Doxorubicin			4.32 ± 1.06

compounds, 6 compounds (**15**, **19**, **25, 32, 33** and **34**) are single-digit micro molar inhibitors. Compound **25** was found to be equally potent (4.8 μM) as compared to the standard drug, doxorubicin (IC_{50} = 4.32 ± 1.06 μM). The substituent fluorine at 4th position in phenyl ring B and any other halogen in aryl ring A increases the potency. Loss of activity was observed when introducing the electron donating groups like methyl and methoxy groups in aryl ring A. Thus, the presence of electron withdrawing substituents in both phenyl rings plays a critical role in cytotoxicity.

Conclusion

A series of *vicinal*-diaryl pyrazole ethyl carboxylate analogs (**14-40**) was synthesized, characterized and evaluated for anti-proliferative activity against PANC-1 cell lines. Six of the synthesized compounds showed significant cytotoxicity against PANC-1 cell line at single digit micro molar level. The presence of halogen substituent(s) either in one or both aryl rings exhibit the cytotoxic activity. Among them, compound **25** was found to be more potent with an IC_{50} of 4.8 μM and also equally effective as observed for standard drug doxorubicin. Further, the structural investigations of these synthesized compounds and biochemical research are needed to reveal its possible mode of action.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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