Middle-East Journal of Scientific Research 17 (7): 926-935, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.17.07.12236

Synthesis Of New Pyrazolidine 3, 5 Dione Derivatives Of Potential Analgesic, Antipyretic And Anti-Inflammatory Activities

[1]Abhishek Tiwari and [2]Anita Singh

[1]Devsthali Vidyapeeth College of Pharmacy, Lalpur,
Rudrapur (U.S. Nagar) Uttarakhand- India
[2]Department of Pharmaceutical Sciences, Kaumaun University,
Bhimtal Campus, Nainital, Uttarakhand- India

Abstract: *Aim*: A novel series of mannich bases 1-(1-((dimethylamino) methyl)-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione QAA-04(K-L) have been synthesized and were evaluated for their analgesic, antipyretic and anti-inflammatory potential. *Method*: Compounds were synthesized by reacting series of 1-(2-oxo-1, 2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione QAA-03(K-L) and diethyl malonate. All the compounds were characterized by their IR, 1H NMR and Mass spectral data. Analgesic potential was evaluated by hot plate and acetic acid induced writhing method, antipyretic potential was evaluated by yeast induced pyrexia and anti-inflammatory potential was evaluated by carrageenan induced rat paw edema method. *Results*: Compounds QAA-04K, QAA-04N, QAA-04O, QAA-04P and QAA-04S were found to possess potent anti-inflammatory activity when compared to the control. Compound QAA-04K was comparable with that of standard drug. Compound QAA-04K, QAA-04N, QAA-04O, QAA-04R and QAA-04S were found to possess a stastically significant analgesic activity and comparable to the standard drug when compared to the control. Whereas QAA-04N, QAA-04O, QAA-04P and QAA-04R, were found to possess a stastically significant analgesic activity and comparable to the standard drug when compared to the control.

Key words: Mannich bases · Pyrazolidine 3, 5 dione · Diethyl malonate · Phenyl hydrazines · Anti-inflammatory · analgesic · Antipyretic activity

INTRODUCTION

Inflammation is a response of the tissue to an infection, irritation or foreign substance [1] and is a part of the host defense mechanism. The inflammatory process involves a series of events that can be elicited by numerous stimuli (e.g. infectious agents, ischemia, antigen-antibody interaction and thermal or other physical injuries) [2]. Almost two decades ago, steroids namely prednisolone, dexamethasone, betamethasone, etc. were considered to be the choicest anti- inflammatory drugs. Owing to the several adverse effects caused by either short-term or long-term steroid therapy, these have been more or less replaced by much safer and better-tolerated non-steroidal anti- inflammatory drugs (NSAID). The seriousness and enormous after effects of steroid therapy necessitated an accelerated research towards the development of NSAIDs since the past two decades [3, 4]. NSAIDs have been highly useful for treating acute, self-limited inflammatory conditions. The development of NSAIDs has helped in understanding the tissue mechanism of inflammation. It is generally agreed that the NSAIDs and analgesic drugs available in the market (aspirin, phenylbutazone, oxyphenbutazone, indomethacin, tenidap, ibuprofen and ketoprofen) are highly acidic in nature and suffer from a common drawback of Gastro intestinal toxicity [5].

2-Quinolones are reported to have antibacterial [6], analgesic [7], anti-inflammatory [8], antifungal [9] and antimalarial activities [10]. Pyrazolidine 3, 5 diones exhibit miticidal, acaricidal, insecticidal, herbicidal actions [11, 12], antipyretic and anti-inflammatory activity [13, 14],

Correspondnig Auhtor: Abhishek Tiwari, Devsthali Vidyapeeth College of Pharmacy, Lalpur, Rudrapur (U. S. Nagar) Uttarakhand- India.

angiotensin receptor antagonists [15,16,17], antioxidant [18], anti depressant, anxiolytic, neuroprotective [19], antimicrobial [20], cytotoxic, antiproliferative [21], antidiabetic [22,23], anti-Alzheimer [24] and anticancer [25]. It is our interest to synthesize some new mannich based 2- quinolones derivatives containing pyrazolidine 3,5 dione moiety and evaluate their analgesic, antipyretic and anti-inflammatory activities. All compounds were tested for their analgesic, antipyretic and anti-inflammatory activities. Compound QAA-04K has shown all the activities, which is comparable with standard drugs.

MATERIALS AND METHODS

Fig. 1 shows the scheme protocol for synthesis. The all reagents used in the present study were of analytical grade and were obtained from the store of Devsthali Vidyapeeth College of Pharmacy. The melting points of the synthesized compounds were determined by open capillary tube method and are uncorrected. The 1H-NMR spectra were recorded at 400 MHz at BRUKER NMR spectrophotometer in $CDCl_3$ and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. The IR spectra were recorded on Shimadzu FTIR 8400S using potassium bromide pellet technique. Mass spectras were recorded on a thermo Finnegan LCQ advantage max ion trap mass spectrometer for title compounds. Spectral data were consistent with the assigned structures.The completions of the reaction were monitored by Thin Layer Chromatographic technique (TLC) on pre-coated silica gel plates using toluene:ethyl acetate (3:2) as the mobile phase. Detection of the spots was done under UV chamber. The test animals were obtained under the IAEC Reference No: 1452/PO/a/11/CPCSEA.

Chemistry: We described here a convenient approach to the preparation of various mannich base derivatives of 1-(1-((dimethylamino) methyl)-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione QAA-04(K-L) in an attempt to find improved analgesic, antipyretic and anti-inflammatory agents.. For the synthesis of the desired compounds Scheme 1 was followed. A cyclization reaction of substituted anilines with diethyl malonoate gave 4-hydroxy-1-methylquinolin-2(1H)-one, which on treatment with posphoryl chloride converted into 4-chloro-1-methylquinolin-2(1H)-one. Subsequently upon halide replacement reaction with phenyl hydrazine gave 1-methyl-4-(phenylhydrazinyl) quinolin-2(1H)-ones, which were cyclized with diethyl malonate gave 1-(1-methyl-2-oxo-1, 2-dihydroquinolin-4-yl)-phenylpyrazolidine-3, 5-diones. Active hydrogen of 2-quinolone was replaced by mannich base reaction, by the reaction of 1-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)-phenylpyrazolidine-3,5-diones, different amines and formaldehyde according to the Mannich reaction.

Synthesis

***(a) General procedure for synthesis of 4-hydroxy-quinolin-2(1H)-one (QAA-01A) [26]*:** A mixture of o- substituted aniline (0.07 mol), diethyl malonate (13mL, 0.1 mol), Dioxane (25mL) and trace of conc. HCl was taken in 250 mL RBF and refluxed at 70-80°C for 3h and then at 260–270°C for 6 h (until the distillation of ethanol stopped), cooled at room temperature and conc. sulfuric acid (20mL) added. The mixture was refluxed at 190-200°C for 1 hr and hot mixture was poured in 500mL of ice cold water with constant stirring (Fig. 1). Separated solid was filtered, dried and recrystallized from ethanol.

***(b)General procedure for synthesis of 4-chloro-quinolin-2(1H)-one [27]*:** In a 150 mL RBF was added the substituted 4-hydroxy-quinolin-2(1H)-one (0.6 miles), $POCl_3$ (9.2g, 0.6 moles) and pyridine (2.37 mL, 0.03 moles). The reaction mixture was heated to 160°C for 2 hours. After cooling, the contents were quenched with cold water (~0°C, 100 mL) and the solution's pH was adjusted to 8–9 with saturated Na_2CO_3 solution (Fig. 1). Solid products were collected after filtration and washing with a small amount of methyl *t*-butyl ether and dried.

***(c) General procedure for synthesis of 4-(2-substitutedphenyl hydrazinyl) quinolin-2(1H)-one (QAA-02K-L) [28, 29, 30, 31]*:** To a suspension of substituted 4-chloro-quinolin-2(1H)-one (0.026mol) in methanol (10mL) added phenyl hydrazine (6ml, 0.05 mol) at room temperature. After stirring, ethanol (20mL) was added and refluxed for 1hr, cooled at room temperature, filtered and washed the solid with diethyl ether (20 mL) and purified from ethanol (Fig. 1).

***(d) General procedure for synthesis of 2-oxo-1, 2-dihydroquinolin-4-yl phenylpyrazolidine -3,5-diones (QAA-03K-L) [32]*:** To a mixture of substituted 4-(phenyl hydrazinyl) quinolin-2(1H)-one (QAA-02K-L) (0.01mol) and diethyl malonate (3mL, 0.015 mol) added ethanol (90mL) and acetic acid (1mL) and refluxed for 5hrs. The reaction mixture was left in open dish for 2-3 hrs (Fig. 1). The solid precipitate formed was filtered, dried and recrystallized from ethanol.

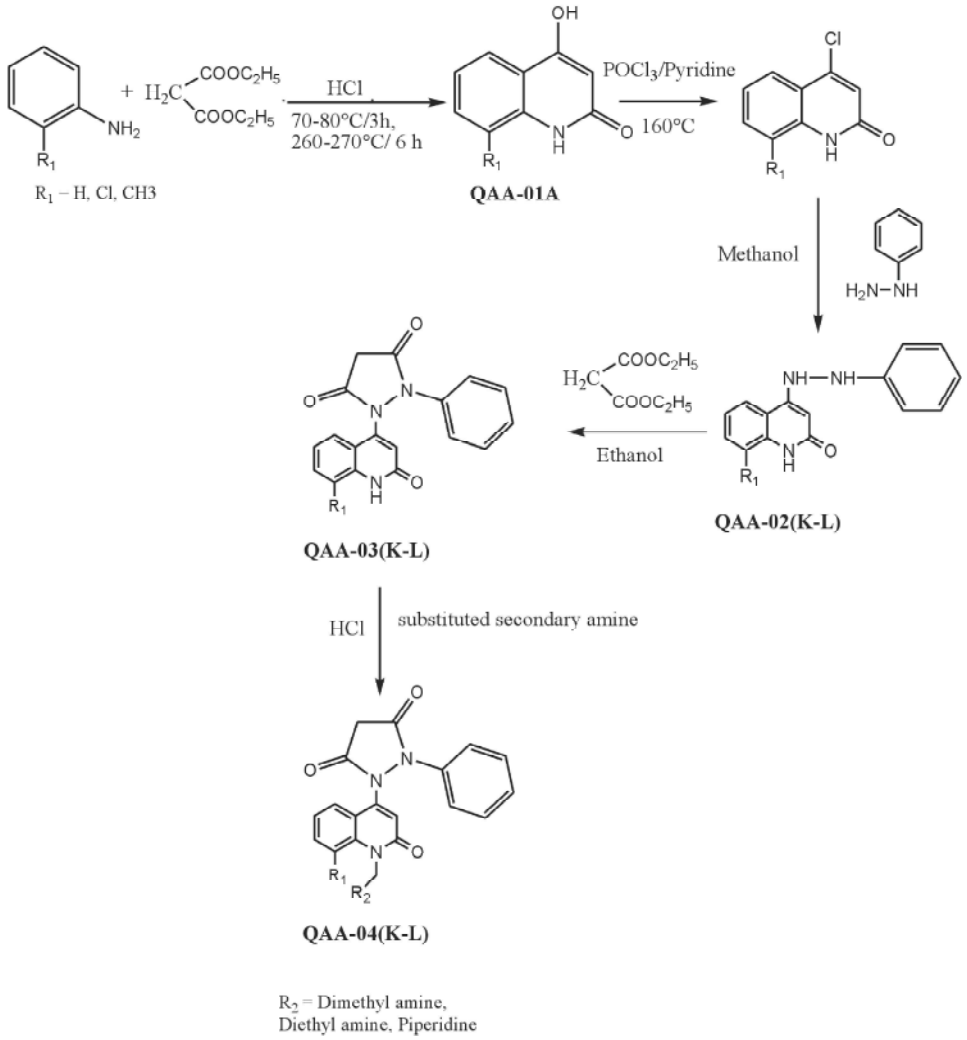

Fig. 1: Protocol for synthesis of 1-(1-((dimethylamino)methyl)-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-diones QAA-04(K-L)

***(e) General procedure for synthesis of 1-(1-((substituted)methyl)-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione [33, 34]*:** A mixture of substituted 2 -oxo-1,2-dihydroquinolin-4-yl phenylpyrazolidine -3,5-diones (QAA-03K-L) (0.004 mol), formaldehyde (5ml,0.004mol), diethylamine (0.5ml, 0.004mol) and HCl (2ml) was heated at reflux in methanol for 2.5 hours (Fig.1). The hot mixture was filtered and the filtrate obtained was cooled in cold water. Crystals obtained were separated by filtration and purified by coloumn chromatography and recrystallized from absolute ethanol.

***1-(1-((dimethylamino) methyl)-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04K)*:** Creamish white solid, yield: 83%, mp 226-229°C; IR (KBr): 3015 (Ar, C-H), 2850 (CH3 alkane), 1670, 1653 (>C=O, quinolone), 1745 (>C=O, pyrazolidine dione), 1175 (C-N) 1H NMR (CDCl3) 1.6-2.7 (6H, N-CH3), 2.9 (2H, N-CH2), 3.72 (2H, pyrazolidine dione), 6.3 (1H C3-H quinolone), 7.1-7.3 (4H, Ar- quinolone), 7.6-7.8 (5H, Ar) m/z 376 (M+).

***1-(8-chloro-1-((dimethylamino) methyl)-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04L)*:** White solid, yield: 74%, mp 194-196°C; IR (KBr): 3045 (Ar, C-H), 2860 (CH3 alkane), 1690 (>C=O, quinolone), 1720 (>C=O, pyrazolidine dione), 1147 (C-N), 762 (C-Cl) 1H NMR (CDCl3): 1.7-2.5 (6H, N-CH3), 2.8 (2H, N-CH2), 3.5 (2H, pyrazolidine dione), 6.7 (1H C3-H quinolone), 7.5-7.9 (3H, Ar- quinolone), 7.2-7.4 (5H, Ar) m/z 410 (M+), 412(M+2).

***1-(1-((dimethylamino) methyl)-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04M)*:** Light yellow crystalline solid, yield: 83%, mp 209-212°C; IR (KBr): 3070, 3035 (Ar, C-H), 2836 (CH3 alkane), 1668 (>C=O, quinolone), 1740 (>C=O, pyrazolidine dione), 1175 (C-N) 1H NMR (CDCl3): 0.9-1.2 (3H, CH3), 1.5-2.4 (6H, N-CH3), 3.2 (2H, N-CH2), 3.6 (2H, pyrazolidine dione), 6.5 (1H C3-H quinolone), 6.9-7.2 (3H, Ar- quinolone), 7.4-7.7 (5H, Ar) m/z 390 (M+).

***1-(8-chloro-1-((diethylamino) methyl)-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04N)*:** White crystalline solid, yield: 83%, mp 246-248°C; IR (KBr): 3056 (Ar, C-H), 2860, 2824 (CH2 alkane), 1696 (>C=O, quinolone), 1725 (>C=O, pyrazolidine dione), 1156 (C-N), 767 (C-Cl) 1H NMR (CDCl3): 1.2-2.4 (10H, C2H5), 2.8 (2H, N-CH2), 3.7 (2H, pyrazolidine dione), 6.9 (1H C3-H quinolone), 7.6-8.0 (3H, Ar- quinolone), 7.2-7.4 (5H, Ar) m/z 438 (M+), 440(M+2).

***1-(1-((diethylamino) methyl)-8-methyl-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04O)*:** Pale yellow crystalline solid, yield: 83%, mp 237-240°C; IR (KBr): 3045, 3012 (Ar, C-H), 2844, 2836 (CH2 alkane), 1690, 1668 (>C=O, quinolone), 1705 (>C=O, pyrazolidine dione), 1160 (C-N), 760 (C-Cl) 1H NMR (CDCl3): 0.9-1.1 (10H, C2H5), 1.2-1.4 (CH3), 1.6-2.7 (10H, C2H5), 2.9 (2H, N-CH2), 3.86 (2H, pyrazolidine dione), 6.4 (1H C3-H quinolone), 7.1-7.3 (3H, Ar-quinolone), 7.6-7.8 (5H, Ar) m/z 418 (M+).

***1-(1-((diethylamino) methyl)-2-oxo-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04P)*:** White amorphous solid, yield: 83%, mp 187-189°C; IR (KBr): 3070 (Ar, C-H), 2847, 2817 (CH2 alkane), 1682, 1670 (>C=O, quinolone), 1715 (>C=O, pyrazolidine dione), 1145 (C-N), 774 (C-Cl) 1H NMR (CDCl3): 1.1-2.6 (10H, C2H5), 3.1 (2H, N-CH2), 3.8 (2H, pyrazolidine dione), 6.2 (1H C3-H quinolone), 7.2-7.7 (4H, Ar- quinolone), 6.9-7.1 (5H, Ar) m/z 405 (M+1).

***1-(2-oxo-1-(piperidin-1-ylmethyl)-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04Q)*:** Off white solid, yield: 83%, mp 215-217°C; IR (KBr): 3089 (Ar, C-H), 2912, 2835 (CH2 alkane), 1675 (>C=O, quinolone), 1760 (>C=O, pyrazolidine dione), 1154 (C-N), 765 (C-Cl) 1H NMR (CDCl3): 1.5-2.4 (8H, piperidine), 3.3 (2H, N-CH2), 3.8 (2H, pyrazolidine dione), 6.8 (1H C3-H quinolone), 7.4-7.6 (4H, Ar- quinolone), 7.7-7.9 (5H, Ar) m/z 417 (M+1).

***1-(8-chloro-2-oxo-1-(piperidin-1-ylmethyl)-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04R)*:** White crystalline solid, yield: 83%, mp 227-229°C; IR (KBr): 3040 (Ar, C-H), 2846 (CH2 alkane), 1675 (>C=O, quinolone), 1740 (>C=O, pyrazolidine dione), 1146 (C-N), 768 (C-Cl) 1H NMR (CDCl3): 1.4-2.0 (8H, piperidine), 2.8 (2H, N-CH2), 3.5 (2H, pyrazolidine dione), 5.8 (1H C3-H quinolone), 6.8-7.3 (3h, Ar-quinolone), 7.5-7.8 (5H, Ar) m/z 450 (M+), 452(M+2).

***1-(8-methyl-2-oxo-1-(piperidin-1-ylmethyl)-1,2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione (QAA-04S)*:** Creamish white solid, yield: 83%, mp 264-267°C; IR (KBr): 3054 (Ar, C-H), 2860 (CH2 alkane), 1674 (>C=O, quinolone), 1715 (>C=O, pyrazolidine dione), 1158 (C-N), 752 (C-Cl) 1H NMR (CDCl3): 0.8-1.3 (3H, CH3), 1.4-2.3 (8H, piperidine), 3.2 (2H, N-CH2), 4.2 (2H, pyrazolidine dione), 5.7 (1H C3-H quinolone), 6.8-7.2 (3H, Ar- quinolone), 7.6-7.8 (5H, Ar) m/z 431 (M+1).

Biological Screenings

Animals: Wistar rats of either sex weighing (150-200 g) were used for studying in-vivo anti-inflammatory and antipyretic activity. Swiss albino mice of either sex weighing 20-25 g were used for in-vivo analgesic activity. Animals were maintained under standard laboratory conditions (24 ± 2°C; relative humidity 60-70%). Study protocol was approved by the institutional Animal Ethics Committee for the Purpose of Control and Supervision on Experiments on Animals (IAEC, Approval No. 1452/PO/a/11/CPCSEA) before experiment. Wiatar Rats and Albino-Swiss mice from Laboratory Animal House Section, Department of Pharmacology, Devsthali Vidyapeeth College of Pharmacy, Lalpur, Rudrapur (U. S. Nagar) were used in the study. The animals were procured from IVRI, Bareilly (U.P.). Minimum of 6 animals were used in each group.

Acute Toxicity Studies: The acute oral toxicity studies were performed to study the acute toxic effects and to determine safest dose of the synthesized compounds. Swiss albino mice of either sex weighing 20-25 g were used for the study. The aqueous solution of compounds were administered orally to different groups of overnight fasted mice at the doses of 30, 100, 300, 1000 and 3000 mg/kg body weight. After administration of the compounds, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hrs.

Table 1: Anti-inflammatory activity of compounds by carrageenin-induced rat paw oedema

	Mean increase in paw volume (mL) ± SEM at time T (h), (% Inhibition)							
Compound tested	1	2	3	4	5	6	7	8
QAA-04K	0.13 ± 0.052*	0.09 ± 0.021*	0.10 ± 0.024*	0.12 ± 0.010***	0.19 ± 0.016***	0.23 ± 0.026***	0.24 ± 0.032***	0.29 ± 0.042***
	(84.33)	(89.15)	(87.95)	(85.54)	(77.10)	(72.28)	(71.08)	(65.06)
QAA-04L	0.30 ± 0.036	0.36 ± 0.015*	0.42 ± 0.027	0.46 ± 0.017	0.52 ± 0.040	0.56 ± 0.036	0.60 ± 0.025	0.64 ± 0.035
	(63.85)	(86.62)	(49.39)	(44.57)	(37.34)	(32.53)	(27.71)	(22.89)
QAA-04M	0.27 ± 0.027*	0.32 ± 0.023	0.36 ± 0.052*	0.45 ± 0.034	0.49 ± 0.021	0.57 ± 0.042	0.68 ± 0.047	0.72 ± 0.045
	(67.46)	(61.44)	(68.67)	(45.78)	(40.96)	(31.32)	(18.07)	(13.25)
QAA-04N	0.16 ± 0.024*	0.17 ± 0.024*	0.26 ± 0.023*	0.34 ± 0.029	0.39 ± 0.027	0.42 ± 0.032	0.47 ± 0.061	0.50 ± 0.029
	(80.72)	(79.51)	(68.67)	(59.03)	(53.01)	(49.39)	(43.37)	(39.75)
QAA-04O	0.18 ± 0.029*	0.19 ± 0.021*	0.21 ± 0.015	0.26 ± 0.018*	0.28 ± 0.042*	0.30 ± 0.034*	0.32 ± 0.041	0.37 ± 0.012
	(78.31)	(77.10)	(64.69)	(68.67)	(66.26)	(63.85)	(61.44)	(55.42)
QAA-04P	0.19 ± 0.017*	0.19 ± 0.029*	0.21 ± 0.018	0.24 ± 0.009	0.26 ± 0.017*	0.28 ± 0.037*	0.29 ± 0.032*	0.30 ± 0.043
	(77.10)	(77.10)	(64.69)	(71.08)	(68.67)	(66.26)	(65.06)	(63.85)
QAA-04Q	0.36 ± 0.018	0.32 ± 0.012	0.47 ± 0.041	0.73 ± 0.012	0.74 ± 0.034	0.76 ± 0.037	0.78 ± 0.032	0.73 ± 0.024
	(54.21)	(61.44)	(43.37)	(12.04)	(10.84)	(8.43)	(6.02)	(12.04)
QAA-04R	0.32 ± 0.014*	0.35 ± 0.016*	0.39 ± 0.062*	0.43 ± 0.012*	0.49 ± 0.034*	0.54 ± 0.037*	0.58 ± 0.034*	0.62 ± 0.062*
	(61.44)	(57.83)	(53.01)	(48.19)	(40.96)	(34.93)	(30.12)	(25.30)
QAA-04S	0.17 ± 0.020*	0.18 ± 0.027*	0.21± 0.042*	0.24 ± 0.014*	0.27 ± 0.025*	0.28 ± 0.036*	0.29 ± 0.034*	0.36 ± 0.021*
	(79.51)	(78.31)	(97.46)	(71.08)	(67.46)	(66.26)	(65.06)	(68.67)
Control	0.83 ± 0.008 (-)	0.83 ± 0.008 (-)	0.83 ± 0.008 (-)	0.81 ± 0.007 (-)	0.80 ± 0.010 (-)	0.75 ± 0.015 (-)	0.75 ± 0.030 (-)	0.70 ± 0.025 (-)
Ibuprofen	0.09 ±0.012**	0.08±0.011**	0.08±0.016** *	0.14 ± 0.007**	0.20±0.022**	0.24 ±0.035**	0.25±0.040**	0.30 ± 0.020**
	(89.15)	(90.36)	(90.36)	(82.71)	(75.0)	(68.0)	(66.66)	(57.14)

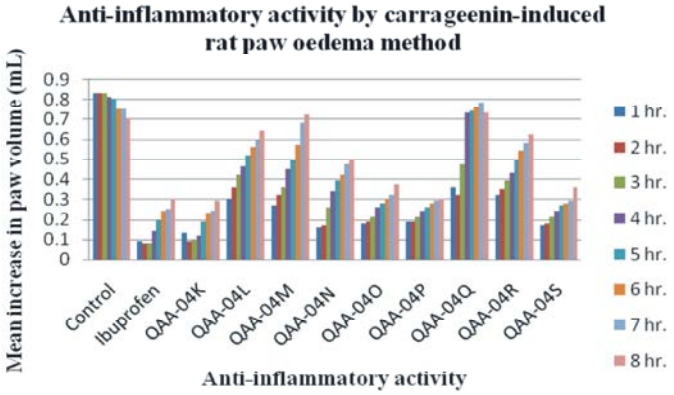

Fig. 2: Comparative effect of synthesized compounds on carrageenin-induced rat paw oedema

Further the animals were under investigation up to a period of one week. 1000 mg/kg body weight was found to be safe and 1500 mg/kg body weight was found to be toxic. So dose was decided 1/10th of the safe dose i.e. 100 mg/ kg body weight.

Anti-Inflammatory Activity [35]: The anti-inflammatory activity of compounds on carrageenin-induced rat paw oedema was determined according to the method described by Winter *et al.*, (1962). The experimental animals were divided into ten groups, each containing five animals. First group received sterile normal saline (0.85% NaCl) assigned as control and the second group received standard drug Ibuprofen (20 mg/kg b.w., p.o.). The 3rd to 10th groups were administered the test compounds (at a dose of 20 mg/kg b.w, suspended in 10 ml/kg of 2% gum acacia) orally. After 30 min of administration of test compounds, 0.1 ml of 1% (w/v) carrageenin was injected subcutaneously in the subplantar region of the left hind paw. The right paw served as a reference to non inflammed paw for comparison. The initial paw volume was measured within 30 sec of the carrageenin injection by plethysmometer. The relative increase in paw volume was measured in control, standard and test compounds at 1, 2, 3, 4, 5, 6, 7 and 8 h after the carrageenin injection. The difference between initial and final readings was taken as the volume of oedema and the percentage inhibition by the compounds was calculated using the formula (Kouadio *et al.*, 2000):

$$\% \, Inhibition = 1 - \left(\frac{dt}{dc}\right) \times 100$$

where *dt* is the difference in paw volume in the test compound-treated group and *dc* the difference in paw volume in the control group. The results of anti-inflammatory activity are shown in Table 1 and Fig. 2.

Table 2: Antipyretc activity of synthesized compounds on yeast induced pyrexia

Treatment	Dose mg/kg	Normal rectal temperature M±SEM	Rectal temp after yeast administration	Rectal temp in °c after drug administration M±SEM		
				1 hr	2 hr	3hr
Control	Saline	34.33±0.20	42.5±0.22	42.3±0.23	41.8±0.25	41.7±0.32
Paracetamol	100	34.7±0.24	41.5±0.17	38.2±0.45*	35.4±0.28**	34.9±0.19*
QAA-04K	100	35.61±0.41	39.8±0.62	38.7±0.359*	38.2±0.21	37.6±0.71
QAA-04L	100	34.93±0.40	43.6±0.23	36.1±0.71**	35.5±0.35**	34.6±0.43**
QAA-04M	100	34.85±0.63	42.5±0.51	40.72±0.40	38.23±0.32	36.36±0.40*
QAA-04N	100	34.66±0.12	41.9±1.42	39.42±0.14	35.24±0.40*	34.8±0.76**
QAA-04O	100	35.49±0.45	38.9±0.56*	37.36±0.23	35.52±0.35*	34.1±0.46**
QAA-04P	100	34.50±0.22	44.5±0.46	38.76±0.36*	36.12±0.34	34.46±0.35**
QAA-04Q	100	34.73±0.34	41.36±0.27	39.12±0.48	37.6±0.53	34.52±0.36**
QAA-04R	100	34.63±0.27	43.6±0.43	40.9±0.72	37.9±0.42	34.13±0.42**
QAA-04S	100	34.43±0.24	41.12±0.48*	39.43±0.65	37.23±0.71	36.22±0.24*

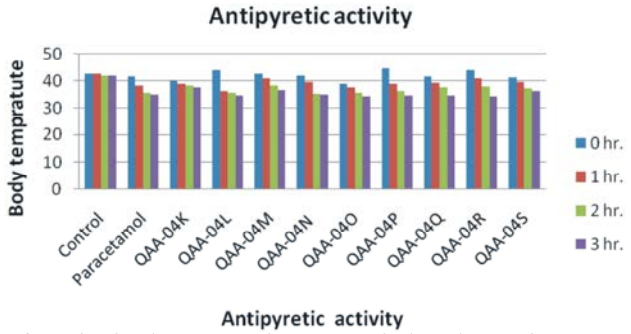

Fig. 3: Comparative effect of synthesized compounds on yeast induced pyrexia

Anti-Pyretic Activity Studies [35]: Rats of Wistar strain of either sex weighing between 170-190g were used. For induction of fever in rats, 20% w/v of brewer's yeast in distilled water was administered by subcutaneous injection. All animals were induced pyrexia by injection of 10 ml/kg of brewer's yeast solution under the skin in between the shoulder blades. The site of the injection was massaged in order to spread the suspension beneath the skin. Basal rectal temperature was measured before the injection of yeast, by inserting digital clinical thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 18 hours after yeast injection. The different groups of febrile rats were orally administered with the respective drugs and rectal temperature was recorded 30, 60, 120, 180 and 300 minutes post treatment. Decrease in rectal temperature post treatment indicated antipyretic effect. The results of antipyretic activity are shown in Table 2 and Fig. 3.

Analgesic Activity

Acetic Acid Induced Analgesic Activity [35]: The analgesic activity of the extracts was evaluated using acetic acid induced writhing method in mice. In this method, acetic acid is administered intra-peritoneally to the experimental animals to induce pain sensation. Albino mice of either sex (25-30 g) were used for the study. In the present study diclofenac sodium was used to as standard. The results of analgesic activity by acetic acid induced writhing model are shown in Table 3 and Fig. 4.

Analgesic Activity by Hot Plate Method [35]: Heat is used as a source of pain. Animals were individually placed on the hot plate maintain at constant temperature (55°C) and the reaction of animals, such as paw licking or jump response was taken as the end response. Analgesic drugs/compounds increases the reaction time. The method was first described by Eddy & Leimbach (A cut off period of 15 sec is observed to avoid damage to the paw). The compounds were dissolved in the Carboxy Methyl Cellulose (0.5% suspension) Control, standard and test compounds were given per orally to the animals and the reaction of time of animals at 30, 60, 90 & 120 min interval was noted on the hot plate after drug administration. The method of Eddy and Leimbach using techno heated plat analgesic apparatus was used. The standard drug Diclofenac Sodium (20 mg/kg) was used reference drug for comparison. The results of analgesic activity by hot plate method are shown in Table 4 and Fig. 5.

Table 3: Analgesic activity of synthesized compounds by acetic acid induced writhing model

Time interval	15 min	30 min	60 min	120 min
Control	6.16±0.32	6.16±0.45	6.16±0.32	6.16±0.54
Standard	6.83±0.16*	11.2±0.65**	13.2±0.25**	14.8±0.46*
QAA-04K	9.00±0.37	13.4±0.34	13.4±0.33*	13.8±0.6**
QAA-04L	5.33±0.42**	13.8±0.29	13.8±0.58*	10.6±0.25***
QAA-04M	6.33±0.47*	10.6±0.36**	10.6±0.64***	12.8±0.13**
QAA-04N	7.83±0.12	12.8±0.25*	12.8±0.39**	13.8±0.65**
QAA-04O	6.23±0.65*	11.42±0.14*	13.4±0.29*	13.6±0.45**
QAA-04P	8.56±0.13	10.82±0.41**	13.8±0.17*	10.5±0.36**
QAA-04Q	7.23±0.42	9.6±0.54***	11.6±0.36***	12.8±0.32**
QAA-04R	9.45±0.36	11.8±0.74*	12.8±0.41**	13.7±0.19*
QAA-04S	7.62±0.17	12.4±0.45*	13.4±0.32*	13.6±0.17*

Table 4: Analgesic activity of synthesized compounds by hot plate method

S.No.	Derivative	Dosage	No. of writhings in 20 min (mean ± S.E.M)	% Analgesic Activity
1	Control	Vehicle	42.3± 0.22	0
2	QAA-04K	20mg/kg	9.1 ± 0.74***	79.18
3	QAA-04L	20mg/kg	12 ± 0.61***	73.34
4	QAA-04M	20mg/kg	10.5 ±1.20	75.88
5	QAA-04N	20mg/kg	11.9 ± 0.67***	71.87
6	QAA-04O	20mg/kg	11.6 ± 0.34**	72.57
7	QAA-04P	20mg/kg	15.2 ± 0.46***	64.06
8	QAA-04Q	20mg/kg	16.7 ± 0.40***	60.51
9	QAA-04R	20mg/kg	14.3 ± 0.28***	66.19
10	QAA-04S	20mg/kg	16. 4± 0.34***	61.23
11	Diclofenac Sodium	20mg/kg	8.1 ± 0.22***	80.84

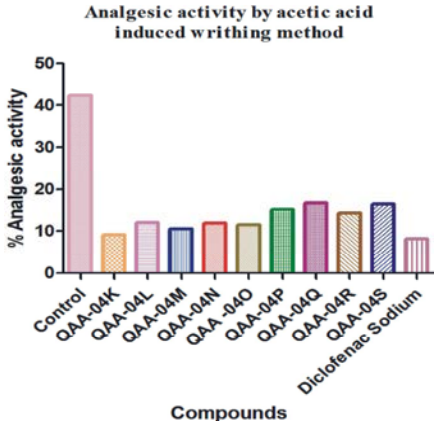

Fig. 4: Comparative effect of synthesized compounds on acetic acid induced writhing in mice

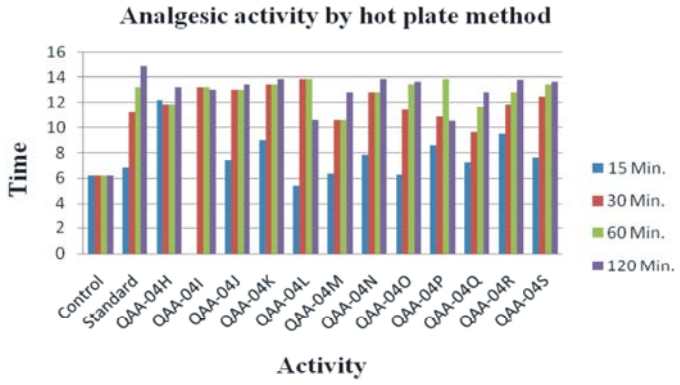

Fig. 5: Comparative effect of synthesized compounds on latency to hot plate test in mice

Results were expressed as means ± S.E.M. Statistical significance was analyzed using the one-way analysis of variance followed by Tukey's Multiple Comparison Test where $p < 0.05$ was accepted to be a significant difference.

RESULTS AND DISCUSSION [36-39]

The present work has clearly demonstrated that mannich base reactions has easily taken place in 2-quinolone moiety, which can be successfully used to synthesize a wide variety of pyrazolidine 3, 5 dione derivatives of pharmaceutical interest. A novel series of compounds QAA-04(K-L) were synthesized and characterized by their IR and NMR data and mass data. All spectral data are in accordance with the assumed structures. In the IR spectra of the compounds, no absorption bands were detected at 3200-3500 cm^{-1}, indicating the absence of an NH group which is an evidence for the addition reaction. The quinolone C=O stretching band was seen at about 1700-1650 cm^{-1} and pyrazolidine 3, 5 dione C=O stretching band was seen at about 1725-1760 cm^{-1}. Aliphatic stretching bands belonging to piperazine ring were appeared at about 2900 cm^{-1} and with methylene appeared about 2850 cm^{-1}. In the 1H-NMR spectra of the compounds, the CH2 protons (pyrazolidine 3,5 dione) of compounds QAA-04(K-L) were seen at about 3.8 ppm as a singlet. The protons of the methyl, ethyl and piperazine ring were seen at a range of 1.2-3.2 ppm. The protons belonging to phenyl ring of pyrazoline 3,5 dione were seen at a range of 7.2-7.8 ppm. The protons belonging to phenyl ring of 2-quinolone were seen in the range of 6.9-7.5 ppm.

Antiinflammatory activities of the compounds were assessed by utilizing carrageenan induced hind paw edema model (Table 1 and Fig. 2). Since the carrageenan edema has been used in the development of indometacin, many researchers adapted this procedure for screening potential anti-inflammatory compounds. Carrageenan-induced edema is a non-specific inflammation maintained by the release of histamine, 5-hydroxytryptamine, kinins and later by prostaglandins. The inhibitory effect of acid NSAIDs, such as indometacin, is usually weak in the first phase (1-2 h), in contrast with their strong inhibition in the second phase (3-4 h). Good inhibition of the second phase of carrageenan-induced edema was observed for the compounds tested, suggesting that they interfere with prostaglandin synthesis and shown. Results of the carrageenan induced rat paw edema (CPE) showed that compounds QAA-04K, QAA-04N, QAA-04O, QAA-04P and QAA-04S were potent anti-inflammatory agents. Compound QAA-04K was as potent as the reference standard.

Antipyretic activities of the compounds were assessed by yeast induced pyrexia model (Table 2 and Fig. 3). Yeast-induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity. There are several mediators for pyrexia and the inhibition of these mediators is responsible for the antipyretic effect. Results of antipyretic activity showed that compounds QAA-04N, QAA-04O, QAA-04P and QAA-04R, were found to possess a significant analgesic activity and comparable to the standard drug.

Analgesic activities of the resulting compounds were investigated by acetic acid induced writhing model (Table 3 and Fig. 4) as well as hot plate method (Table 4 and Fig. 5) which are well established methods of testing the analgesic activity of compounds and sufficiently sensitive to detect the effect of analgesics which were compared with diclofenac sodium. Results of analgesic activity via acetic induced writhing method showed that compound QAA-04K, QAA-04L, QAA-04M, QAA-04N and QAA-04O were found to possess a significant analgesic activity and comparable to the standard drug. Results of analgesic activity via hot plate model showed that compound QAA-04K, QAA-04N, QAA-04O, QAA-04R and QAA-04S were found to possess a significant analgesic activity and comparable to the standard drug.

CONCLUSION

From the above study it was concluded that, various derivatives of bases 1-(1-((dimethylamino) methyl)-2-oxo-1, 2-dihydroquinolin-4-yl)-2-phenylpyrazolidine-3,5-dione were successfully synthesized and tested for their analgesic, anti-inflammatory and antipyretic activities. Mannich base derivatives of 2-quinolones have shown good activities. Compounds QAA-04K, QAA-04N and QAA-04O showed significant analgesic, anti-inflammatory and antipyretic properties.

ACKNOWLEDGMENT

The authors are thankful to Devsthali Vidyapeeth College of Pharmacy, Lalpur, Rudrapur (U. S. Nagar),

Sophisticated Analytical Instrument Facilities, Punjab University, Chandigarh for providing IR, ^{1}HNMR and mass spectra.

REFERENCES

1. Turner, R.A., 1965. Screening Methods in Pharmacology, Academic Press, New York, 1: 152.
2. Paul, A.I., 1996. Analgesic-antipyretic and Anti-inflammatory Agents and Drugs Employed in the Treatment of Gout. In Goodman and Gilmans, The Pharmacological Basis of Therapeutic, Perry, B.M. and Raymond, W.R., Eds, McGraw Hill, New York, 9th ed, pp: 618.
3. Ashutoshkar, 2003. Medicinal Chemistry New Age International Publishers, New Delhi, 2nd ed, pp: 329.
4. Kant, G.A., S.C. Parate and Chaturvedi, 2005. QSAR study of substituted 3, 5-di-tertbutyl-4-hydroxy styrene; a series with anti inflammatory activity. Indian J Pharm Sci, 67(1): 116119.
5. Mattison, N., A.G. Trimple and I. Lasangna, 1998. New drying development in the United States, 1963 through 1988. Cli Pharmacol Ther, 43: 290.
6. Andriole, V.T., 2006. The Quinolones. 2nd ed. Academic Press San Diago, California (USA), 2: 24-25.
7. Jia, C.S., Y.W. Dong, S.J. Tu and G.W. Wang, 2007. Microwave-assisted solvent-free synthesis of substituted 2-quinolones, Tetrahedron, 63: 892-897.
8. Marcaccini, S., R. Pepino, M.C. Pozo, S. Basurto, M.G. Valverde and T. Torroba, 2004. One-pot synthesis of quinolin-2-(1*H*)-ones via tandem Ugi–Knoevenagel condensations. Tetrahedron Letters, 45(21): 3999-4001.
9. Al-Huniti, M.H., M.M. El-Abadelah, J.A. Zahra, S.S. Sabri and A. Ingendoh A., 2007. Heterocycles [h] fused onto 4-oxaquinoline-3-carboxlic acid, Part IV. convenient synthesis of substituted hexhydro [1,4] thiazepino [2,3-h] quinoline-9-carboxilic acid and its tetrahyderoquino[7,8-h] benzothiazipine homolog. Molecule, 12: 1558-68.
10. Kalkhambkar, R.G., G.M. Kulkarni, W.S. Hwang and C.S. Lee, 2007. Analytical Sciences, 23: 145-146.
11. Takahashi, *et al*., 2001. 4-Aryl-4-substituted pyrazolidine-3, 5-dione derivatives. U S Patent, Jan, 30: 6180568.
12. Scheiblich, S., 1995. Preparation of herbicidal 1, 4-di-heteroaryl pyrazolidin-3, 5-diones. PCT Int Appl WO 94 22, 854, Chem Abstr, 122: 81389w.
13. Baccolin, G., M. Gianelli, 1995. N-Alkenyl-3, 5-Pyrazolidinediones from Ketone Hydrazones, PCl_3 and Malonic acid. Tetrahedron Lett, 51(34): 9487-92.
14. Goda F.E., A.R. Maarouf and E.R. El-Bendary, 2003. Synthesis and antimicrobial evaluation of new isoxazole and pyrazole derivatives. Saudi Pharm J., 11(3): 111-17.
15. Barnes, B.J., R.A. Izydore, A.E. Eakin and I.H. Hall, 2001. Induction of $Tmolt_4$ Leukemia Cell Death by 3, 3-Disubstituted-6, 6-pentamethylene-1,5-diazabicyclo [3.1.0] hexane- 2, 4-diones: Specificity for Type II Inosine 5'-Monophosphate Dehydrogenase. J. Pharmacol. Exp. Ther., 298(2): 790-96.
16. Cauvin, C., B.L. Bourdonnec, B. Norberg, J.P. Henichart and F. Durant F., 2001. Pyrazolidine-3, 5-dione angiotensin-II receptor antagonists. Acta Cryst, C, 57: 1330-32.
17. Fretz, *et al*., 2007. Pyrazolidinedione derivatives. U S Patent, Feb 15: 0037846 A1.
18. Tandon, V.K., D.B. Yadav, A.K. Chaturvedi and P.K. Shukla, 2005. Synthesis of (1, 4)-naphthoquinono-[3, 2-c]-1H-pyrazoles and their (1, 4)-naphthohydroquinone derivatives as antifungal, antibacterial and anticancer agents. Bioorganic and Medicinal Chemistry Letters, 15: 3288-91.
19. Shen, D.M., *et al*., 2011. Discovery of novel, potent, selective and orally active human glucagon receptor antagonists containing a pyrazole core. Bioorganic and Medicinal Chemistry Letters, 21(1): 76-81.
20. Zheng, L.W., J. Zhu, B.X. Zhao, Y.H. Huang, J. Ding and J.Y. Miao, 2010. Synthesis, crystal structure and biological evaluation of novel 2-(5-(hydroxymethyl)-3-phenyl-1H-pyrazol-1-yl)-1-phenylethanol derivatives. European Journal of Medicinal Chemistry, 45(12): 5792-5799.
21. Chioua, M., A. Samadi, E. Soriano, O. Lozach, L. Meijer and J.C. Marco, 2009. Synthesis and biological evaluation of 3, 6-diamino-1H-pyrazolo[3, 4-b]pyridine derivatives as protein kinase inhibitors. Bioorganic and Medicinal Chemistry Letters, 19(16): 4566-4569.
22. Bomburn, *et al*., 2004. Alkylidene pyrazolidenedione derivatives. U S Patent, Nov, 4: 0220188 A1.
23. Litvinov, Y.M., A.A. Shestopalov, L.A. Rodinovskaya and A.M. Shestopalov, 2009. New Convenient Four-Component Synthesis of 6-amino-2, 4-dihydropyrano[2, 3-c]pyrazol-5-carbonitriles and one pot synthesis of 6'-aminospiro[(3H)-indol-3,4'-pyrano[2, 3-c]pyrazol]-(1H)-2-on-5'-carbonitriles. Journal of Combinatorial Chemistry, 11(5): 914-919.

24. Li, Y., D. Hong, P. Lu and Y. Wang, 2011. Synthesis of pyrazoles from 2-azidoacrylates and hydrazonyl chlorides. Tetrahedron Letters, 52(32): 4161-4163.
25. Ragavan, R.V., V. Vijayakumar and N.S. Kumari, 2010. Synthesis and antimicrobial activities of novel 1, 5-diaryl pyrazoles. European Journal of Medicinal Chemistry, 45(3): 1173-1180.
26. Fiala, W. and Stadlbauer, 1993. Neucleophilic chlorination of 3-formyl-4-hydro-quinolin-2(1H)-2-ones. J. Prakt. Chem., 335: 128-134.
27. Tiwari, A., A. Singh and V. Tiwari, 2011. Synthesis and Biological Activity of Quinolone Derivatives. Journal of Pharmacy Research, 4(4): 1063-1064.
28. El-Emary, T.I., 2006. Synthesis and Biological Activity of Some New Pyrazolo [3, 4-b] pyrazines. J. Chin. Chem. Soc., 53: 391-401.
29. Deshmukh, M.B., S.S. Jagtap, S.A. Deshmukh, 2006. Solvent free accelerated synthesis of 2-hydrazinobenzothiazole derivatives using microwave. J. Indian Chem. Soc., 83: 1055-57.
30. Vankataramana, C.H.S., A. Singh, A. Tiwari and V. Tiwari, 2010. Synthesis of phenyl hydrazine substituted benzimidazole derivatives and their biological activity. Int J. Pharm. Sci. Res., 1: 32.
31. Tiwari, A., V. Tiwari, C.H.S. Vankataramana and V. Madhvan, 2011. Synthesis of Novel Pyrazolidine-3, 5-Dione Substituted Benzimidazole Derivatives and their biological activity, Asian J. Chem., 23(3): 1179-1182.
32. Tiwari, A. and A. Singh, 2013. Synthesis and evaluation of possible mechanism of anti nociceptive potential of novel 2-quinolone fused 3, 5-pyrazolidinedione derivatives in experimental animal models. Ovidius University Annals of Chemistry, 24(1): 5-12.
33. Sharma, V., K.V. Sharma, 2006. Synthesis and Biological some 3, 5-Diaryl- 1-benzo thiazolopyrazoline Derivatives, Reaction of Chalcones with 2- Hyrazino benzothiazoles", E-Journal of Chemistry. E-Journal of Chemistry, 6(2): 356-384.
34. Reddy, B.A., 2010. Synthesis, Characterization and Biological Evaluation of 1, 2-Disubstituted Benzimidazole Derivatives using Mannich Bases. J. Chem., 7(1): 222-226.
35. Kuppusamy, S., T.P. Paramasivan, M. Doraisamy and R. Melani, 2009. Synthesis, analgesic and anti-inflammatory activities of bis (indolyl) methanes. Ind J. Chem., 48B: 267-272.
36. Koksal, M., N. Gokhan, E. Kupeli, E. Yesilada and H. Erdogan, 2010. Analgesic and Antiinflammatory Activities of Some New Mannich Bases of 5-Nitro-2-Benzoxazolinones. Arch Pharm. Res., 30(4): 419-424.
37. Abou-Deif, M.H., M.A. Rashed, M.A.A. Sallam, E.A.H. Mostafa and W.A. Ramadan, 2013, Characterization of Twenty Wheat Varieties by ISSR Markers, Middle-East Journal of Scientific Research, 15(2): 168-175.
38. Kabiru Jinjiri Ringim, 2013. Understanding of Account Holder in Conventional Bank Toward Islamic Banking Products, Middle-East Journal of Scientific Research, 15(2): 176-183.
39. Muhammad Azam, Sallahuddin Hassan and Khairuzzaman, 2013. Corruption, Workers Remittances, Fdi and Economic Growth in Five South and South East Asian Countries: A Panel Data Approach Middle-East Journal of Scientific Research, 15(2): 184-190.