Original research article

SYNTHESIS AND BIOLOGICAL EVALUATION STUDIES OF NOVEL BENZO-1,3-DIAZINE DERIVATIVES AS ANTI-INFLAMMATORY AGENTS

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Abstract

A series of substituted 6-bromo-3-(3-chloro-2-oxo-4-arylazetidin-1-yl)-2-methylquinazolin-4(3H)-onehas been synthesized and evaluated for their biological activity. The title compounds (G_1-G_{10}) were prepared by the reaction of 5-bromo anthranilic acid with acetic anhydride to form 6-bromo-2-methyl-4H-benzo[1,3]oxazin-4-one which upon treatment with hydrazine hydrate in the presence of anhydrous pyridine form 3-amino-6-bromo-2-methylquinazolin-4(3H)-one. This resulting intermediate undergoes Schiff reaction with different aromatic aldehyde followed by reflux with chloroacetylchlorideand triethylamine. Ten different quinazoline derivatives (G_1-G_{10}) were synthesized. Structural assignments of these compounds have been made by elemental analysis, FTIR, ^{1}HNMR and Mass spectral data and the purity of the compounds was determined by TLC.All synthesized compounds have been tested for their anti-inflammatory activityby using diclofenac sodium as a standard drug.Most of the compounds showed a moderate degree of potent anti-inflammatory activity. The study concluded that the compound G_3& G_8were found to exhibit significant anti-inflammatory activity when compared to standard drug.

Keywords: Anti-inflammatory activity, Azetidine,5-bromo anthranilic acid, Diclofenac sodium, Schiff base

Introduction

Quinazoline is a fused six-member aromatic ring (a benzene ring and a pyrimidine ring are fused). Quinazoline is a fused bicyclic compound earlier known as benzo 1, 3-diazine[1]. It was first prepared in the laboratory in 1903 by Gabriel. Although its derivative were known much earlier. The name benzo 1.3-diazine (German: Chinazolin) was first proposed for this compound by weddige on observing that this was isomeric with the compounds cinnoline and quinoxaline. Paal and Bush suggested the numbering of quinazoline ring system, which is currently used[2-4]. The other less commonly used names for this ringsystem are 'phenmiazine' and 5, 6-benzopyrimidine. However, the name quinazoline is now universally accepted. Benzo 1,3-diazine derivatives, which belong to the N-containing heterocyclic compounds, have caused universal concerns due to their widely and distinct biopharmaceutical activities. Researchers have already determined many therapeutic activities of benzo 1.3-diazine derivatives, including anti-cancer[5-8], anti-inflammation[9-10], anti-bacterial[11-14], analgesia[9,13], anti-viral[15], anti-cytotoxin[16], anti-spasm[13,17], anti-tuberculosis[18], anti-oxidant[19], anti-malarial[20], anti-hypertension[21], anti-obesity[22], anti-psychotic[23], anti-diabetes[24] etc. Medicinal chemists synthesized a variety of benzo 1.3-diazine compounds with different biological activities by installing various active groups to the benzo 1.3-diazine moiety using developing synthetic methods and the potential applications of the benzo 1.3-diazine derivatives in fields of biology, pesticides and medicine have also been explored. benzo 1.3-diazine(quinazoline) derivatives have attracted much attention for their various biological and medicinal properties. For example, they act as the potent tyrosine kinase and cellular phosphorylation inhibitors[25] and they are also used as ligands for benzodiazepine and GABA receptors in the central nervous system[26] or as DNA binders[27-29]. Some of them show remarkable activity as anticancer[30], antiviral[31] and antitubercular agents[32-33]. Molecules containing the benzo 1.3-diazineunit have been popular drugs. For example, Erlotinib is used in the treatment of several types of tumors[34],Prazosin acts as an adrenergic blocker[35] and Iressa as an epidermal growth factor receptor inhibitor was approved by the Food and Drug Administration in USA for the treatment of lung cancer[36]. Encouraged by the diverse biological activities of benzo 1.3-diazineHeterocyclic compounds, it was decided to prepare a new series of benzo 1.3-diazine derivatives. Literature survey revealed that incorporation of different groups in benzo 1.3-diazine Heterocyclic ring enhanced anti-inflammatory activity.

Recently quinazolinone derivatives seek great attention of researchers in organic and medicinal chemistry due to their prompt biological activities. Encouraged by the therapeutic diversity of quinazolinone containing moiety and the comparative ease of convertibility of anthranilic acid to quinazolinone, we took up the synthesis of certain novelquinazolinone from 5-bromo anthranilic acid and evaluated their anti-inflammatoryactivity.

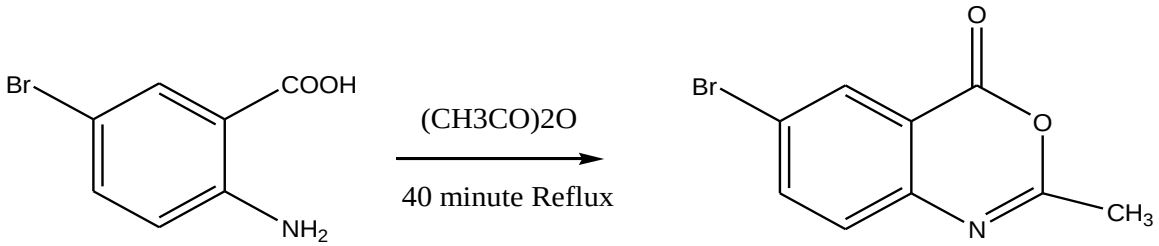
EXPERIMENTAL:

Materials and Methods:

All the chemicals used in the synthesis of the intermediates and final derivatives were of A.R grade and procured from the Merck and LOBA chemicals. All the synthesized benzo 1.3-diazine derivatives were characterized by melting point determination using Veergo digital melting point apparatus in open capillary tubes and were uncorrected.

IR Spectra were recorded using Perkin Elmer FTIR spectrophotometer using KBr pellets techniques and ^{1}HNMR spectra of the synthesized compounds in deuteriated DMSO were recorded on BRUKER AVANCE II 400MHz NMR Spectrometer instrument using TMS as the internal standard. Mass Spectra were recorded using LC-MSD-Tranp-SL2010A SHIMADZU using Dimethylsulphoxide (DMSO) as solvent. TLC was performed using silica gel GF_{254} coated platesof0.25 mm thickness. Petroleum ether &Ethyl acetate (1:2) were used as solvent system and iodine vapoursas visualizing agent.

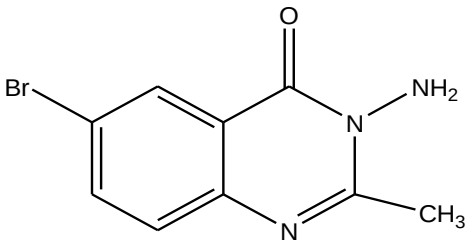
Scheme of Synthesis:



2-amino-5-bromobenzoic acid

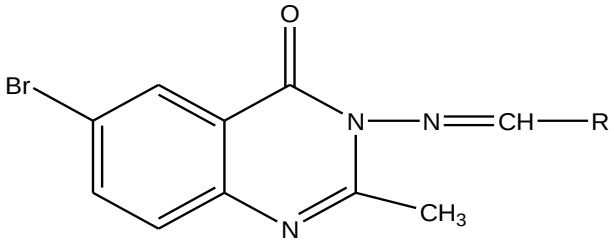
6-bromo-2-methyl-4*H*-benzo[1,3]oxazin-4-one

6 hrs Reflux | NH2.NH2.H2O, Anhydrous Pyridine



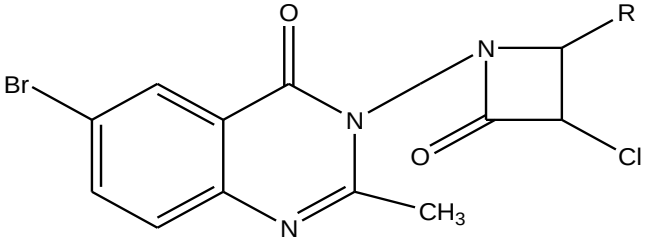
3-amino-6-bromo-2-methylquinazolin-4(3*H*)-one

8-10 hrs Reflux | R-CHO, Schiff Reaction



3-(substituted benzylideneamino)-6-bromo-2-methylquinazolin-4(3*H*)-one

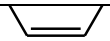
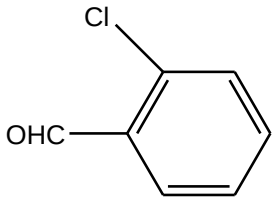
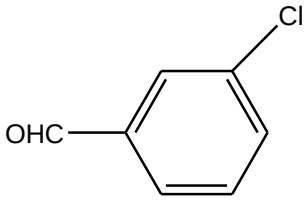
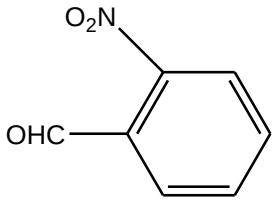
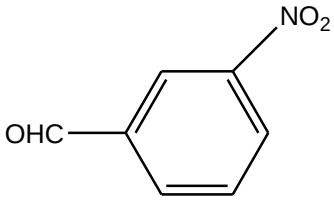
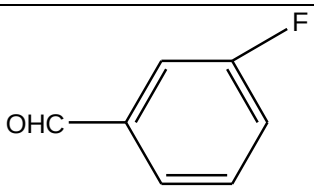
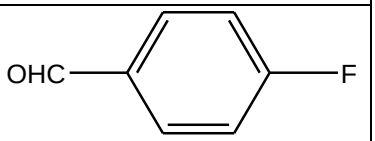
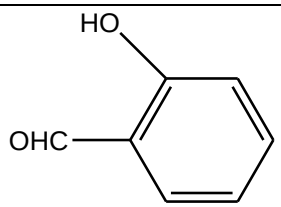
8 hrs Reflux | Cl.CH2COCl, (C2H5)3N

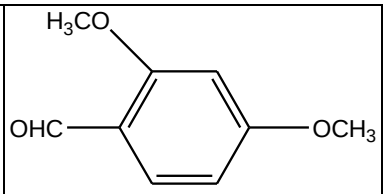
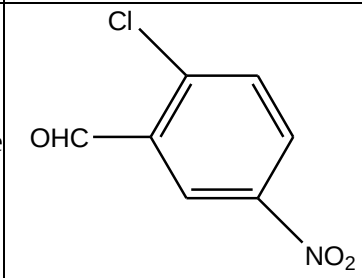


6-bromo-3-(3-chloro-2-oxo-4-arylazetidin-1-yl)-2-methylquinazolin-4(3H)-one

R = Different Aromatic Aldehyde

Table 1: LIST OF VARIOUS AROMATIC ALDEHYDE

S. No.	Compounds Code	Substituted Aromatic Aldehyde (Ar)	Structure of Aromatic Aldehyde (Ar)
1	G_1	Benzaldehyde	
2	G_2	o-Chloro Benzaldehyde	
3	G_3	m-Chloro Benzaldehyde	
4	G_4	o-Nitro Benzaldehyde	
5	G_5	m-Nitro Benzaldehyde	
6	G_6	m-Fluoro Benzaldehyde	
7	G_7	p-Fluoro Benzaldehyde	
8	G_8	o-Hydroxy Benzaldehyde	

9	G_9	o,p-DimethoxyBenzaldehyde	
10	G_{10}	2-Chloro-5-Nitro Benzaldehyde	

The Experimental Work Comprises in Four Steps.

Step-I: Synthesis of 6-bromo-2-methyl-4H-benzo [1, 3] oxazin-4-one from 5-bromo anthranilic acid.

Step-II: Synthesis of 3-amino-6-bromo-2-methylquinazolin-4(3H)-one.

Step-III: Synthesis of 3-(substituted benzylideneamino)-6-bromo-2-methylquinazolin-4(3H)one. (Preparation of Schiff base derivatives)

Step-IV: Synthesis of 6-bromo-3-(3-chloro-2-oxo-4-arylazetidin-1-yl)-2-methylquinazolin-4(3H)-one.

Step-I: General Procedure for the synthesis of 6-bromo-2-methyl-4H-benzo [1, 3] oxazin-4-

onefrom 5-bromo anthranilic acid (Intermediate-I):

5-bromo anthranilic acid (5 gm, 0.023 moles) allowed to mix with acetic anhydride (100ml, 0.979 moles) and refluxedfor 40 min. Then the solution was cool to room temperature and excess acetic anhydride removed under reduce pressure and the crude product was allowed to filter and dried. The dried crude product then recrystallized from cyclohexane.

Yield: 78.51%, M.P.: 167-169^{o}C.

Step-II: General Procedure for the synthesis of 3-amino-6-bromo-2-methylquinazolin-4-(3H)-one (Intermediate-II):

3-amino-6-bromo-2-methylquinazolin-4(3H)-one was prepared by adding dropwise a solution of hydrazine hydrate (3.204 ml, 0.1 mol) in anhydrous pyridine (25 ml) to cold solution of 6-bromo-2-methyl-4H-benzo[1,3]oxazin-4-one(12 gm, 0.05 mol) in anhydrous pyridine (25 ml), with constant stirring. When the addition completed, the resultant reaction mixture was stirred

vigorously for 30 min at room temperature and subsequently heated under reflux for 6 h under anhydrous reaction conditions. It was allowed to cooled at room temperature and poured into ice cold water containing dilute hydrochloric acid on standing for 1 h, solidification occur which was allowed to settle down. It was then filtered off, washed repeatedly with water and dried in vacuum.

Yield: 74.39%, M.P.: 202-204^{o}C.

Step-III: Synthesis of 3-(substituted benzylideneamino)-6-bromo-2-methylquinazolin-4(3H)-one:-

(Preparation of Schiff base derivatives)

To a solution of the appropriate substituted benzaldehyde (0.001 mol) in ethanol (15 ml) were add to the 3-amino-6-bromo-2-methylquinazolin-4(3H)-one (0.001 mol) and a few drops of acetic acid (0.05 mol). The reacting mixture will then refluxed for 8-10 hrs and the course of the reaction was monitor by TLC [petroleum ether/ethyl acetate (*V*/*V*=1:2)] to its completion. The reacting mixture is then allowed to cool. The crude product were recrystallized from 95% ethanol to give title compounds.

Step-IV: Synthesis of 6-bromo-3-(3-chloro-2-oxo-4-arylazetidin-1-yl)-2-methylquinazolin-4(3H)-one:-

(General Procedure)

A mixture of Schiff-base sofquinazolinone(0.01 mol),1,4-dioxan (50 ml), Chloroacety lchloride(0.01 mol) and triethylamine(0.01 mol) refluxed on water bath for 8 hrs. The resultant mixture then transferred to the beaker and ice cold water was added to it. The separated solid was then filtered off, washed with water and recrystallized from ethanol to give different substituted benzo 1.3-diazinecompounds [G_1 to G_{10}].

Spectral Analysis of Different Derivatives:

G_1: 6-bromo-3-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-methylquinazolin-4(3H)-one:

Offwhite colored solid, Molecular formula: $C_{18}H_{13}BrClN_3O_2$, Molecular weight:418.67, Yield: 76.55%, M.P.: 126-128^{o}C, $R_f$value: 0.68, **FT-IR (KBr, cm^{-1}):** 3052.41 (=C-H Str.), 2862.23 (C-H in CH_3 Str.), 1572.09 (C=C Str.), 1648.58 (C=O Str.), 1268.65 (C-N Str.), 727.25 (Ar C-H Bend.), 525.31 (C-Br Bend.), 1598.60 (C=N Str.), 1743.17 (C=O in β-lactum), 584.28 (C-Cl Bend.).**^{1}H-NMR (400 MHz, DMSO, δ ppm):** 7.18-8.23 (m, 8H, Ar H), 0.92 (s, 3H, CH_3), 5.38 (d, 1H, CH), 4.98 (d, 1H, CH).**Elemental Analysis, % found (% required):** C 51.58 (51.64);

H 3.08 (3.13);Br19.02 (19.09);Cl8.40 (8.47);N10.01 (10.04); O 7.56 (7.64).

G_2:6-bromo-3-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin4(3H)-

one:

Yellowish brown colored solid, Molecular formula: $C_{18}H_{12}BrCl_2N_3O_2$, Molecular weight: 453.12, Yield: 67.38%, M.P.: 132-134^oC, R_f value: 0.86, **FT-IR (KBr, cm^{-1}):**3023.33 (=C-H Str.), 2925.12 (C-H in CH_3 Str.), 1593.33 (C=C Str.), 1673.42 (C=O Str.), 1284.83 (C-N Str.), 739.40 (Ar C-H Bend.), 534.25 (C-Br Bend.), 1592.45 (C=N Str.), 1752.14 (C=O in β-lactum), 628.54 (C-Cl Bend.).**^{1}H-NMR (400 MHz, DMSO, δ ppm):** 7.06-8.14 (m, 8H, Ar H), 0.94 (s, 3H, CH_3), 5.43 (d, 1H, CH), 5.12 (d, 1H, CH).**Mass Spectra: m/z:** 454.92 (M^{+2}). **Elemental Analysis, % found (% required):**C 47.58 (47.71); H 2.62 (2.67); Br 17.58 (17.63); Cl 15.58 (15.65);N 9.22 (9.27); O 7.02 (7.06).

G_3:6-bromo-3-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin4(3H)-

one:

Creamish yellow colored solid, Molecular formula: $C_{18}H_{12}BrCl_2N_3O_2$, Molecular weight: 453.12, Yield: 70.18%, M.P.: 136-138^oC, R_f value: 0.82, **FT-IR (KBr, cm^{-1}):**3045.64 (=C-H Str.), 2874.45 (C-H in CH_3 Str.), 1587.23 (C=C Str.), 1686.71 (C=O Str.), 1320.25 (C-N Str.), 724.75 (Ar C-H Bend.), 629.52 (C-Br Bend.), 1601.25 (C=N Str.), 1754.75 (C=O in β-lactum), 678.28 (C-Cl Bend.).**^{1}H-NMR (400 MHz, DMSO, δ ppm):** 7.01-8.18 (m, 8H, Ar H), 0.89 (s, 3H, CH_3), 5.42 (d, 1H, CH), 5.16 (d, 1H, CH). **Mass Spectra: m/z:** 466.57 (M^{+2}). **Elemental Analysis,**

% found (% required):C 47.10 (47.71); H 2.61 (2.67); Br 17.59 (17.63); Cl 15.55 (15.65); N 9.24 (9.27); O 7.01 (7.06).

G_4:6-bromo-3-(3-chloro-2-(2-nitrophenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin-4(3H)-

one:

Pale yellow colored solid, Molecular formula: $C_{18}H_{12}BrClN_4O_4$, Molecular weight: 463.67, Yield: 68.48%, M.P.: 129-131^oC, $R_f$value: 0.73, **FT-IR (KBr, cm^{-1}):**3012.54 (=C-H Str.), 2942.43

(C-H in CH_3 Str.), 1621.57 (C=C Str.), 1657.27 (C=O Str.), 1338.43 (C-N Str.), 730.38 (Ar C-H Bend.), 645.74 (C-Br Bend.), 1604.76 (C=N Str.), 1662.25 (C=O in β-lactum), 720.15 (C-Cl Bend.),1521.47 (C-NO_2Str.).**^{1}H-NMR (400 MHz, DMSO, δ ppm):** 7.32-8.16 (m, 8H, Ar H), 0.91 (s, 3H, CH_3), 5.41 (d, 1H, CH), 5.14 (d, 1H, CH). **Mass Spectra: m/z:**465.18 (M^{+2}).**Elemental Analysis, % found (% required):**C 46.57 (46.63); H 2.58 (2.61); Br 17.18 (17.23); Cl7.57 (7.65); N 12.02 (12.08); O 12.72 (13.80).

G_5: 6-bromo-3-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin-4(3H)-

one:

Pale red colored solid, Molecular formula: $C_{18}H_{12}BrClN_4O_4$, Molecular weight: 463.67, Yield: 74.24%, M.P.: 155-157^oC, $R_f$value: 0.76, **FT-IR (KBr, cm^{-1}):**3062.48 (=C-H Str.), 2961.32 (C-H in CH_3 Str.), 1637.27 (C=C Str.), 1642.35 (C=O Str.), 1276.08 (C-N Str.), 729.58 (Ar C-H Bend.), 667.20 (C-Br Bend.), 1607.35 (C=N Str.), 1767.45 (C=O in β-lactum), 815.64 (C-Cl Bend.),1536.31 (C-NO_2Str.).**^{1}H-NMR (400 MHz, DMSO, δ ppm):** 7.34-8.12 (m, 8H, Ar H), 0.96 (s, 3H, CH_3), 5.48 (d, 1H, CH), 4.18 (d, 1H, CH).**Elemental Analysis, % found (% required):**C 46.60 (46.63); H 2.56 (2.61); Br 17.18 (17.23); Cl7.58 (7.65); N 12.01 (12.08); O 13.75 (13.80).

G_6:6-bromo-3-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin-4(3H)-

one:

Light red colored solid, Molecular formula: $C_{18}H_{12}BrClFN_3O_2$, Molecular weight: 436.66, Yield: 76.36%, M.P.: 142-144^oC, $R_f$value: 0.68, **FT-IR (KBr, cm^{-1}):**3034.26 (=C-H Str.), 2912.53

(C-H in CH_3 Str.), 1648.21 (C=C Str.), 1634.73 (C=O Str.), 1272.45 (C-N Str.), 728.96 (Ar C-H Bend.), 681.25 (C-Br Bend.), 1609.28 (C=N Str.), 1748.49 (C=O in β-lactum), 766.18 (C-Cl Bend.),1241.07 (C-F Bend.**).^{1}H-NMR (400 MHz, DMSO, δ ppm):**6.82-8.20 (m, 8H, Ar H), 0.95(s, 3H, CH_3), 5.52 (d, 1H, CH), 5.16 (d, 1H, CH). **Mass Spectra: m/z:** 438.25 (M^{+2}).**Elemental Analysis, % found (% required):**C 49.50 (49.51); H 2.71 (2.77); Br 18.74 (18.30); Cl8.08 (8.12); F 4.31(4.35); N 9.56 (9.62); O 7.20 (7.33).

G_7:6-bromo-3-(3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin-4(3H)-

one:

Greyish red colored solid, Molecular formula: $C_{18}H_{12}BrClFN_3O_2$, Molecular weight: 436.66, Yield: 69.82%, M.P.: 143-145^oC, R_f value: 0.69, **FT-IR (KBr, cm^{-1}):**3058.16 (=C-H Str.), 2885.12 (C-H in CH_3 Str.), 1579.25 (C=C Str.), 1628.41 (C=O Str.), 1312.52(C-N Str.), 732.15 (Ar C-H Bend.), 577.45 (C-Br Bend.), 1605.87 (C=N Str.), 1746.75 (C=O in β-lactum), 834.31 (C-Cl Bend.),1356.23 (C-F Bend.**).^{1}H-NMR (400 MHz, DMSO, δ ppm):**6.95-8.17 (m, 8H, Ar H), 0.93 (s, 3H, CH_3), 5.46 (d, 1H, CH), 5.17 (d, 1H, CH). **Mass Spectra: m/z:**438.40 (M^{+2}).**Elemental Analysis, % found (% required):**C 49.49 (49.51); H 2.72 (2.77); Br 18.22 (18.30); Cl 8.07 (8.12); F 4.32(4.35); N 9.54 (9.62); O 7.21 (7.33).

G_8:6-bromo-3-(3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin-4(3H)-

one:

Dark Brown colored solid, Molecular formula: $C_{18}H_{13}BrClN_3O_3$, Molecular weight: 434.67, Yield: 66.30%, M.P.: 156-158^oC, $R_f$value: 0.82, **FT-IR (KBr, cm^{-1}):**3074.16 (=C-H Str.), 2984.42

(C-H in CH_3 Str.), 1609.51 (C=C Str.), 1679.27 (C=O Str.), 1306.25 (C-N Str.), 737.21 (Ar C-H Bend.), 591.35 (C-Br Bend.), 1594.75 (C=N Str.), 1764.70 (C=O in β-lactum), 742.83 (C-Cl Bend.),3249.85**(**C-OH Str.**).^{1}H-NMR (400 MHz, DMSO, δ ppm):**6.71-8.19 (m, 8H, Ar H), 0.96 (s, 3H, CH_3), 5.43 (d, 1H, CH), 5.19 (d, 1H, CH). **Elemental Analysis, % found (% required):**C 49.66 (49.74); H 2.94 (3.01); Br 18.32 (18.36); Cl8.09 (8.16); N 9.58 (9.67);
O 10.96 (11.04).

G_9:6-bromo-3-(3-chloro-2-(2,4-dimethoxyphenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin

4(3H)-one:

Pale Brown colored solid, Molecular formula: $C_{20}H_{17}BrClN_3O_4$, Molecular weight: 478.72, Yield: 71.84%, M.P.: 182-184^oC, $R_f$value: 0.78, **FT-IR (KBr, cm^{-1}):**3084.60 (=C-H Str.), 2936.67

(C-H in CH_3 Str.), 1632.27 (C=C Str.), 1683.24 (C=O Str.), 1316.25 (C-N Str.), 734.36 (Ar C-H Bend.), 614.15 (C-Br Bend.), 1591.15 (C=N Str.), 1758.38 (C=O in β-lactum), 594.62 (C-Cl Bend.).**^{1}H-NMR (400 MHz, DMSO, δ ppm):**6.26-8.27 (m, 8H, Ar H), 0.97 (s, 3H, CH_3), 5.56 (d, 1H, CH), 5.20 (d, 1H, CH), 3.71 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3). **Mass Spectra: m/z:** 480.61 (M^{+2}).**Elemental Analysis, % found (% required):**C 50.09 (50.18); H 3.52 (3.58);

Br 16.63 (16.69); Cl7.37 (7.41); N 8.71 (8.78); O 13.29 (13.37).

G_{10}:6-bromo-3-(3-chloro-2-(2-chloro-5-nitrophenyl)-4-oxoazetidin-1-yl)-2-methylquinazolin-

4(3H)-one:

Creamish Brown colored solid, Molecular formula:$C_{18}H_{11}BrCl_2N_4O_4$, Molecular weight: 498.11, Yield: 64.96%, M.P.:163-165^oC, R_f value: 0.89, **FT-IR (KBr, cm^{-1}):**3091.25 (=C-H Str.), 2957.14 (C-H in CH_3 Str.), 1599.14 (C=C Str.), 1639.47 (C=O Str.), 1293.78 (C-N Str.), 731.05 (Ar C-H Bend.), 582.94 (C-Br Bend.), 1596.48 (C=N Str.), 1769.25 (C=O in β-lactum), 642.41 (C-Cl Bend.),1547.45 (C-NO_2Str.).**^{1}H-NMR (400 MHz, DMSO, δ ppm):** 7.46-8.23 (m, 8H, Ar H), 0.91 (s, 3H, CH_3), 5.49 (d, 1H, CH), 5.20 (d, 1H, CH). **Elemental Analysis, % found (% required):**C 43.32 (43.40); H 2.16 (2.23); Br 16.01 (16.04); Cl 14.14 (14.23); N 11.18 (11.25);

O 12.76 (12.85).

Biological Study:

Evaluation of Anti-Inflammatory Activity[37, 38]**:**

Adult albino rats of both sexes weighing between 120 and 150 g were used. Rats were uniformly hydrated by giving 3 ml water/rat through gastric inoculation to reduce variability to oedema response. Animals were divided into 18 groups each of five animals. The control groupwas given saline solution containing few drops of Tween 80. Diclofenac sodium (50 mg/kg) was taken as standard drug for comparison and compounds under examination (100 mg/kg) were suspended in distilled water by the aid of few drops of Tween 80 and were given orally 1 hr before induction of inflammation. Induction of inflammation was performed by S.C. injection of 50 µl of 1% carrageenan-sodium gel into the sub-plantar region of the right hind paw. The dorso-ventral diameter (thickness) of the right and left hind paw of each rat was measured using a pair of dial thickness gauge callipers accurateto 0.001 cm 0.5, 1, 2 and 3 hr after induction of inflammation. The left hind paw diameter served as a control for the degree of inflammation in the right hind paw. The percentage of anti-inflammatory activity (% inhibition of inflammation) was calculatedaccording to the following equation:

% inhibition = (Wc – Wt / Wc) × 100

Wt: is the mean increase in paw thickness in rats treated with the tested compounds.

Wc: is the mean increase in paw thickness in control group.

RESULTS AND DISCUSSION:

Chemistry: All the novel benzo 1.3-diazinederivatives were synthesized, purified and separated by using column chromatography or recrystallization method. Synthesized compounds were characterized by using Elemental analysis, FT-IR, ^{1}HNMR and Mass Spectrometric studies. The integration curves fully support the orientation of protons in the analyzed compounds. Furthermore, all the compounds demonstrated the characteristic chemical shifts for the benzo 1.3-diazine nucleus. Additionally, synthesized compounds were analyzed by mass spectra and indicated no difference in the fragmentation pattern among the set of synthesizedseries.

Anti-Inflammatory Activity: The anti-inflammatory activity was assessed by using Carrageenan-induced rat paw edema using Diclofenac sodium as the reference drug. The anti-inflammatory activity data was obtained as thickness of edema at 0.5 hr, 1 hr, 2 hr and 3 hr intervals and expressed in % inhibition as shown in Table 2 and 3. Compounds $G_3$and

G_8showed excellent anti-inflammatory activity as 74.46% and 72.34% inhibition respectively at 3rdhr, which were nearby 78.72 % inhibition of the standard Diclofenac sodium used and also greater than the other benzo 1.3-diazinederivatives.

Table 2: Oedema thickness of control, diclofenac sodium and tested compounds.

Compounds	Oedema thickness (mm) ± SEM			
	0.5 hr	1 hr	2 hr	3 hr
Control	0.380 ± 0.020	0.410 ± 0.022	0.440 ± 0.021	0.470 ± 0.021
Diclofenac sodium	0.160 ± 0.008	0.150 ± 0.006	0.120 ± 0.004	0.100 ± 0.005***
G_1	0.240 ± 0.013	0.220 ± 0.013	0.200 ± 0.011	0.170 ± 0.012**
G_2	0.250 ± 0.014	0.210 ± 0.013	0.190 ± 0.015	0.160 ± 0.010***
G_3	0.200 ± 0.007	0.180 ± 0.009	0.150 ± 0.008	0.120 ± 0.007***
G_4	0.260 ± 0.016	0.250 ± 0.015	0.230 ± 0.011	0.200 ± 0.014**
G_5	0.290 ± 0.007	0.280 ± 0.008	0.280 ± 0.008	0.270 ± 0.006**
G_6	0.280 ± 0.011	0.260 ± 0.012	0.250 ± 0.011	0.230 ± 0.013**
G_7	0.300 ± 0.004	0.290 ± 0.005	0.270 ± 0.003	0.260 ± 0.004**
G_8	0.220 ± 0.009	0.190 ± 0.006	0.170 ± 0.008	0.130 ± 0.008***
G_9	0.240 ± 0.010	0.220 ± 0.011	0.180 ± 0.012	0.160 ± 0.010***
G_{10}	0.270 ± 0.008	0.240 ± 0.007	0.220 ± 0.009	0.190 ± 0.006**

Values are expressed as mean±SEM of five animals in each group. **Statistically significant (P<0.05).

***Statistically significant (P<0.01)

Table-3: % Inhibition of Compounds on Carrageenan induced Paw RatPaw Edema

Compounds	Oedema inhibition (%)			
	0.5 hr	1 hr	2 hr	3 hr
Control	-	-	-	-
Diclofenac sodium	57.89	63.41	72.72	78.72***
G_1	36.84	46.34	54.54	63.82**
G_2	34.21	48.78	56.81	65.95***
G_3	47.36	56.09	65.90	74.46***
G_4	31.57	39.02	47.72	57.44**
G_5	23.68	31.70	36.36	42.55**
G_6	26.31	36.58	43.18	51.06**
G_7	21.05	29.26	38.63	44.68**
G_8	42.10	53.65	61.36	72.34***
G_9	36.84	46.34	59.09	65.95***
G_{10}	28.94	41.46	50	59.57**

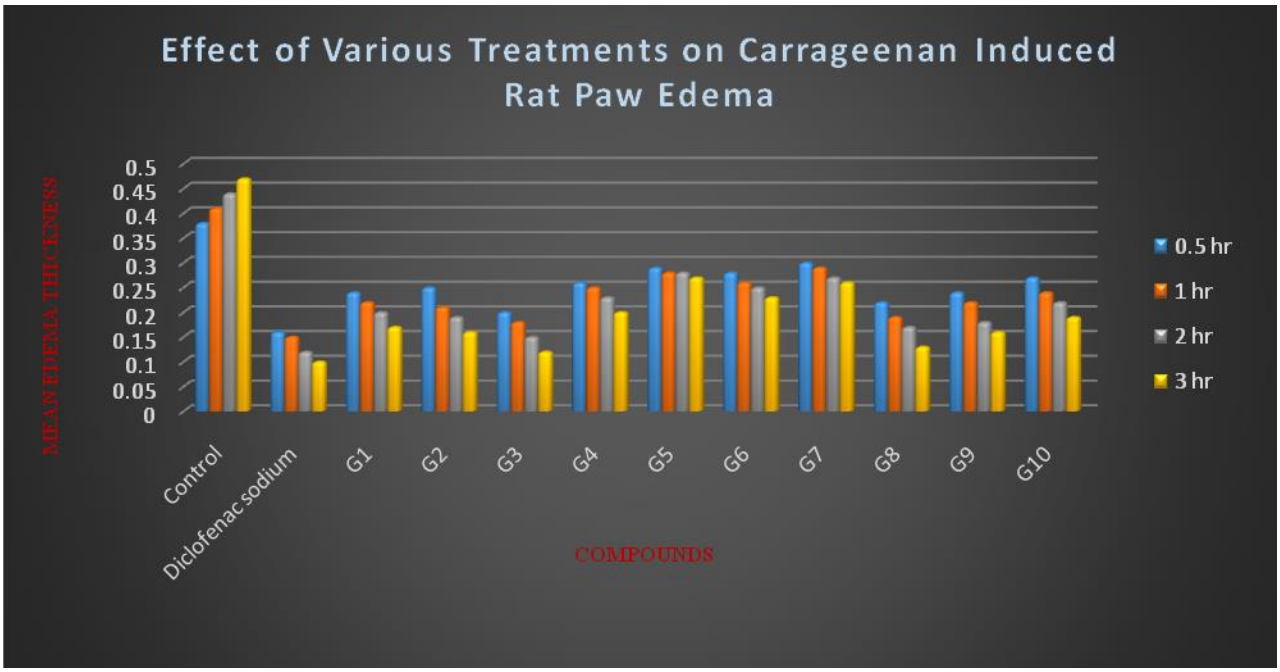


Figure 1- Effect of various treatments on mean edema thickness in carrageenan induced ratpawedema

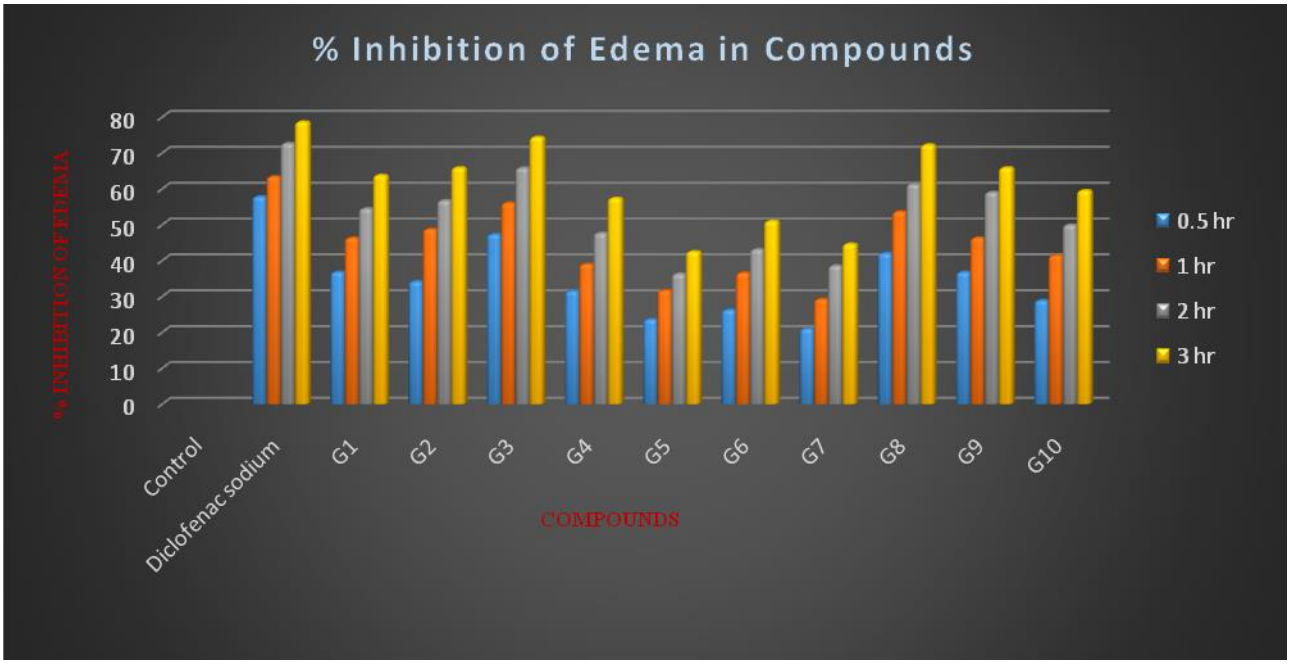


Figure 2- Effect of Various treatments on mean edema thickness in carrageenan induced ratpawedema

CONCLUSION:

The main focus of this research work was to synthesize novel series of benzo 1.3-diazinederivatives, purify, characterize and evaluate their anti-inflammatory activity.From the results, it can be concluded that the modified benzo 1.3-diazine show

significant biological evaluation as anti-inflammatory agents. However, further evaluation of benzo 1.3-diazine will be undertaken, concerning the structural arrangements inring for anti-inflammatoryactivity.

ACKNOWLEDGEMENT:

The authors arethankful to Department of Pharmaceutical Chemistry, IndoreInstitute of Pharmacy, Indore for providing chemicals and facilities to conduct research work.

CONFLICT OF INTEREST: The authors declared no conflict of interest.

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