Iranian Journal of Organic Chemistry Vol. 7, No. 3 (2015) 1605-1612

Synthesis of novel pyrimidines, pyrimidopyrimidines and their oxygen substituted hydroxylamine derivatives as potential pharmacological interest

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Received: July 2015; Revised: July 2015; Accepted: July 2015

Abstract: In this investigation, the synthesis of benzoxazole based oxygen substituted hydroxylamine functionalized pyrimidines **4a**, **4b** and pyrimido[4,5-d]pyrimidines **7a**, **7b**, **9**, & **10** is achieved by using 1-(1,3-benzoxazol-2-yl)guanidine as a starting material via multistep routes. Structures of newly synthesized compounds were confirmed by analytical, spectral data & chemical tests. *In-vivo* anti-inflammatory activity of targeted compounds was tested against carrageenan-induced paw edema in albino rats. Half of the compounds exhibited appreciable activity when compared to reference drug.

Keywords: Guanidinobenzoxazole, Pyrimidines, Pyrimidopyrimidines, Imidoxy derivatives, Biological activity.

Introduction

Multi-component reactions (MCRs) have emerged as powerful tools in organic synthesis. By providing arrays of novel heterocyclic frameworks, these strategies often facilitate identification of new lead structures in the area of drug discovery [1]. Benzoxazole derivatives are frequently targeted in medicinal chemistry research due to their prominent biological importance. In view of considerable therapeutic potential, benzoxazole derivatives possess insecticidal [2] anti-tumor [3], antibacterial [4, 5], anticancer [6], antiviral [7, 8], antimicrobial [9], anti-inflammatory and antioxidant [10] properties. They also lead to industrial applications in the field of textiles [11], dyes and pigments [12]. Recently, ^{123}I-labeled pyridyl benzoxazole (PBOX) derivatives are reported [13] as SPECT probes for imaging Ab plaques *in- vivo.* Pyrimidine is an important class of nitrogen-containing heterocyclic compounds as it constitutes vital structural framework of organic molecules like DNA and RNA; it also plays a lead role in the biosynthesis of specific proteins [14]. Compounds containing pyrimido[4,5-d]pyrimidine scaffold that have been found to show pharmacophoric properties, such as antiplatelet [15], tyrosine kinase inhibitor [16], antihistaminic, antiasthmatic [17], antifolate [18], antibacterial [19], anti-inflammatory [20], hepatoprotective [21], antiviral [22] etc. Many aminooxy compounds have been tested for their ability to inhibit the growth of the malaria parasite *Plasmodium falciparum* in-vitro [23]. Furthermore, drug research and development has led to the discovery of new biologically active agents including imidoxy (N–O linkage), compounds such as succinimidoxy and phthalimidoxy [24-28].

Recent report in our laboratory has focused on the synthesis of biological active heterocyclic compounds from modified guanidine. Keeping in mind the synthetic and medicinal applications and as a continuation of our previous work [29-34], we

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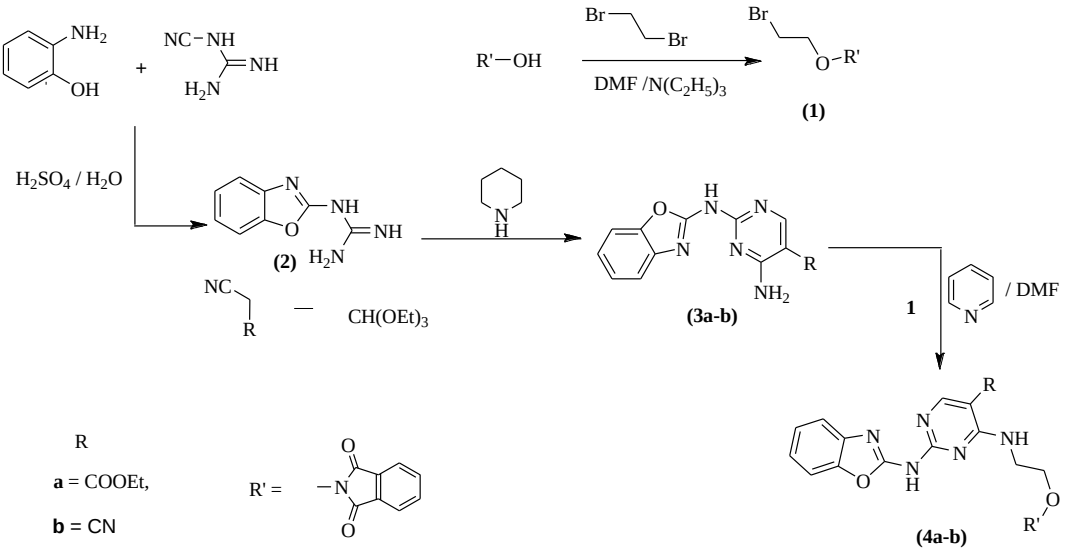
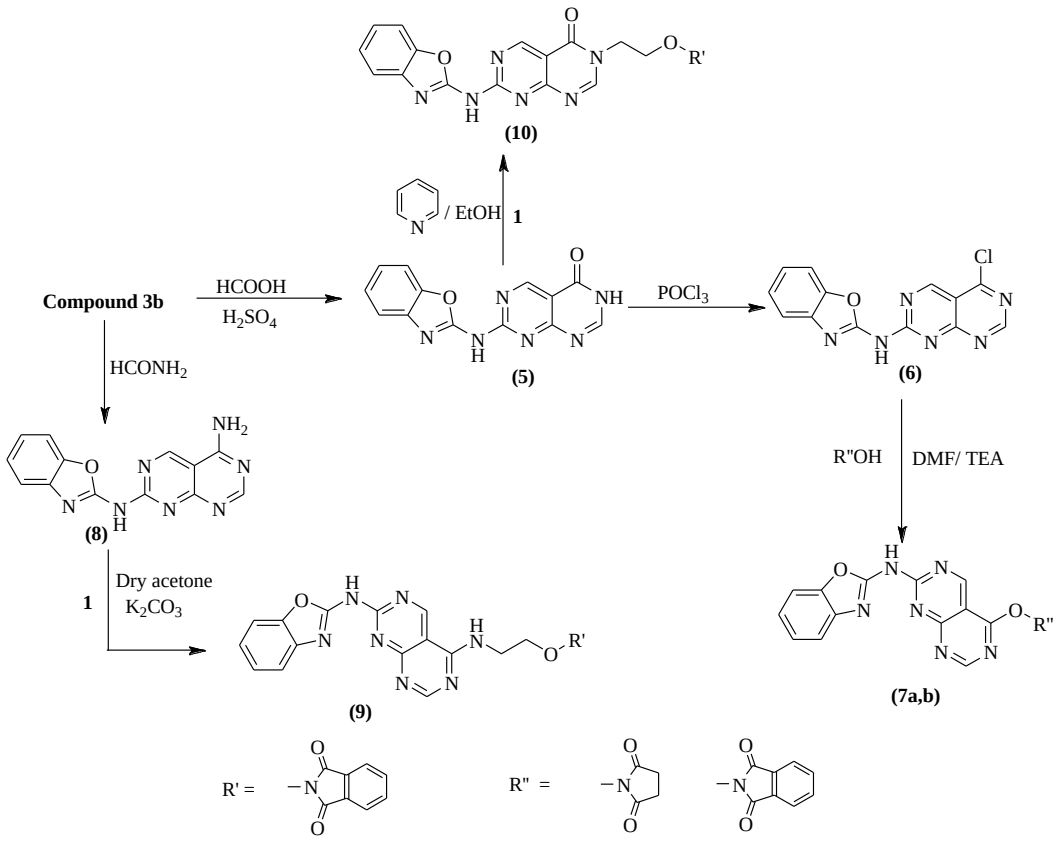
envisioned our approach toward the synthesis of a novel series of oxygen-substituted hydroxylamine derivatives by incorporating the above active pharmacophores in a single molecular framework with a potential spectrum of anti-inflammatory activity.

Results and discussion

In present paper, substituted-(1*H*-benzo[d]imidazol-2-yl)amino-pyrimidine derivatives **3a, b** are obtained by three component reaction between 2-guanidinobenoxazole **(2)**, triethyl orthoformate and different reactive methylene compounds. The structures of these compounds were confirmed by appearance of two peaks at range between 3469-3326 cm^{-1} due to NH_2 group and 1737, 2148 cm^{-1} due to C=O and CN streaching of pyrimidine ring respectively, in IR spectrum. Similarly, a broad signal appeared at 9.62, 11.98 δ for NH group while methine (=CH) group of pyrimidine ring observed singlet at 8.90, 8.52 δ in ^{1}H NMR spectrum. In next step, compounds **3a, b** were condensed with phthalimidoxy ethyl bromide **(1)** in the presence of DMF media using pyridine as base to give compounds **4a, b**. It elucidated by appearance of new vibration bands at 1198, 1215 cm^{-1} and 1365, 1370 cm^{-1} due to CH_2 –N-CO and N-O group respectively, in IR spectrum and appearance of new triplets at 3.76, 3.79 δ and 3.36, 3.39 δ due to O-CH_2 and N-CH_2 of ethoxyphthalimide moiety in ^{1}H NMR spectrum **(**Scheme **1).** In advance research, compound **3b** reacts with excess amount of formic acid using H_2SO_4 as a catalyst to give compound **5**. The structure of this compound was assigned on the basis of spectral data: at 1695 cm^{-1} for C=O stretching in IR spectrum and characteristic peak at 9.56 δ due to CONH group in ^{1}H NMR spectrum. Treatment of this compound with $POCl_3$ yielded compound **6**, which reacts with N-hydroxysuccinimide/phthalimide to obtain the corresponding final compounds **7a, b.** The presence of a C=O peak at 1698, 1700 cm^{-1} and C-O stretching at 1004, 1010 cm^{-1} in IR spectrum confirmed the formation of the final compounds **7a, b**. On the other hand, compound **5** was condensed with phthalimidoxy ethyl bromide **(1)** to give compound **10**. It was confirmed by disappearance of NH band in IR spectrum and appearance of two new triplets at 3.35 δ due to N-CH_2 and 3.85 δ due to O-CH_2 of ethoxyphthalimide moiety in ^{1}H NMR spectrum. In another route compound **3b** treated with formamide to give compound **8**. Formation of this was confirmed by disappearance of IR band for the C≡N group and also protons of NH_2 group present in **3b** were also present in **8**. Further replacement of reactive hydrogen takes place by nucleophilic substitution of ethoxyphthalimide group in the presence of base K_2CO_3 in acetone media using phthalimidoxy ethyl bromide **(1)** to obtain targeted compound **9.** A formation of this product was elucidated by disappearance of NH_2 peak in IR spectrum (Scheme **2**). The mass spectrum also supports the proposed structure by viewing molecular ion peaks of synthesized compounds. Addition confirmation of succinimidoxy/phthalimidoxy group attachment was achieved by usual chemical test including fluorescence formation.



Scheme 1: Synthesis of pyrimidine derivatives **4a** and **4b**.



Scheme 2: Synthesis of pyrimidopyrimidine derivatives **7a**, **7b**, **9** and **10**.

Conclusion

New benzoxazole based oxygen substituted hydroxylamine functionalized pyrimidines and pyrimido[4,5-d]pyrimidines were synthesized and screened for their anti-inflammatory activity. Out of 6 compounds screened, three compounds, i.e., **7a**, **7b** and **10** showed significant anti-inflammatory activity and they can be developed as potent chemotherapeutic agents.

Experimental

Material and methods:

All chemicals were commercially procured and were used without further purification. Melting points were determined in open capillary tube and are uncorrected. FT-IR spectra were recorded with a Perkin-Elmer BX spectrum on KBr pellets and NMR were recorded on a Bruker DRX-400 MHz spectrometer with DMSO-d_6 /$CDCl_3$ as solvent using TMS as an internal standard. The mass spectra were recorded on Joel SX-102 (EI) model. Purity of synthesized compounds was checked by TLC using silica gel-G plates, n-hexane - ethyl acetate as developing solvent and the spots were exposed in iodine chamber. Elemental analysis was done on "Heraeus Rapid Analyser". 2-(2-bromoethoxy)-1H-isoindole-1,3(2H)-dione **(1)** and 1-(1,3-benzoxazol-2-yl)guanidine **(2)** were synthesized by reported method [35, 36]. 2-hydroxy-1*H*-isoindole-1,3(2*H*)-dione and 1-hydroxypyrrolidine-2,5-dione has been purchased by commercial resources. *In-vivo* anti-inflammatory activity was studied at B. N. Institute of pharmaceutical science, Udaipur, India.

Synthesis of 1-(1,3-benzoxazol-2-yl)guanidine 2:

2-aminophenol (0.05mol) was dissolved on heating in 50 mL of 10% sulfuric acid and cyanoguanidine (0.075mol) was added. The reaction mixture was refluxed for 1 h and then 15 mL of 50% NaOH solution was added and heated for further 20 minutes. The reaction mixture was cooled and the obtained solid was collected by filtration, washed with water, dried. The prepared compound was sufficiently pure and used without further purification.

Yield 94%; mp 186-188°C; IR (KBr): 3454, 3242, 3035, 1610, 1442 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ ppm : 6.98 (s, 1H, NH), 3.45 (s, 2H, NH_2), 7.11 - 7.23 (m, 4H, Ar-H), 11.24 (s, 2H, br exchangeable NH); MS *m/z* 176 [M^+].

General procedure for the synthesis of compounds 3a & 3b:

A solution of compound **2** (0.01mol) and an equivalent molar ratio of the malanonitryle or ethylcynoacetate in triethyl orthoformate (25 mL), in the presence of few drops of piperidine were heated under stirred reflux for 1.5 h. The excess solvent was removed by distillation under reduced pressure and the residue was left to cool. The precipitated solid product was collected by filtration, washed with ethanol, dried and recrystallized from DMF.

Ethyl 4-amino-2-(1,3-benzoxazol-2-ylamino) pyrimidine-5-carboxylate 3a:

Yield 72%; mp 190-192°C; IR (KBr): 3485, 3329, 3154, 3042, 2856, 1737, 1614, 1416, 1046 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.56 (s, 2H, NH_2), 1.28 (t, 3H, CH_3), 4.26 (q, 2H, OCH_2), 7.07–7.52 (m, 4H, Ar-H), 8.90 (s, 1H, CH), 9.62 (s, 2H, br exchangeable NH); MS: m/z 299 [M^+]; Anal. calcd. For $C_{14}H_{13}N_5O_3$: C, 56.18; H, 4.38; N, 23.40; Found: C, 56.08; H, 4.46; N, 23.32.

4-amino-2-(1,3-benzoxazol-2-ylamino)pyrimidine-5-carbonitrile 3b:

Yield 78%; mp 274-276°C; IR (KBr): 3469, 3326, 3192, 3031, 2148, 1622, 1434, 1188 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ : 3.39 (s, 2H, NH_2), 7.11–7.51 (m, 4H, Ar-H), 8.52 (s, 1H, CH, pyrimidine ring), 11.98 (2H, br s, exchangeable NH). MS *m/z* 252 [M^+]; Anal. calcd. For $C_{12}H_8N_6O$: C, 57.14; H, 3.20; N, 33.32; Found: C, 57.20; H, 3.12; N, 33.41.

General procedure for the synthesis of compounds. 4a & 4b:

Compound **3a** or **3b** (0.003mol) and Phthalimidoxy ethyl bromide **1** (0.003mol) were dissolved in DMF (15mL). Pyridine was added to this solution as a base. The reaction mixture was refluxed for 17 -20 h in around bottom flask. Excess of solvent was distilled of under reduced pressure .The reaction mixture was cooled to room temperature and poured into crushed ice. Products were filtered, washed with cold water, dried and recrystallized from ethanol**.**

2-(1,3-benzoxazol-2-ylamino)-4-({2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)oxy]ethyl}amino) pyrimidine -5-carboxylic acid ethyl ester 4a:

Yield 64%; mp 179-181°C; IR (KBr): 3356, 3025, 2858, 1735, 1690, 1610, 1370, 1215, 1453, 1052 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.22–7.65(8H, m, Ar-H), 9.12(s, NH), 8.15 (s, 1H, CH), 5.32 (t, 1H, $NHCH_2$), 4.28 (q, 2H, OCH_2 ester), 1.86 (t, 3H, CH_3), 3.76 (t, 2H, OCH_2), 3.39 (t, 2H, NCH_2): ^{13}C NMR (400 MHz, $CDCl_3$): δ 38.22, 58.12, 70.24, 74.67, 75.36, 118.74, 121.14, 124.16, 126.49, 128.66, 129.98, 130.55, 130.96, 133.41 134.66, 138.25, 140.22, 143.19, 150.20, 154.21, 164.29, 164.31, 169.44; MS: m/z 488 [M^+]; Anal. calcd. For $C_{24}H_{20}N_6O_6$: C, 59.01; H, 4.13; N, 17.21; Found: C, 56.09; H, 4.21; N, 17.34.

2-(1,3-benzoxazol-2-ylamino)-4-({2-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)oxy]ethyl}amino) pyrimidine-5-carbonitrile 4b:

Yield 65%; mp 201-203°C; IR (KBr): 3359, 3018, 2143, 1698, 1622, 1365, 1198, 1448, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.20–7.69(8H, m, Ar-H), 9.04 (s, NH), 8.16 (s, 1H, CH), 5.34 (t, 1H, $NHCH_2$), 3.79 (t, 2H, OCH_2), 3.33 (t, 2H, NCH_2): ^{13}C NMR (400 MHz, $CDCl_3$): δ 58.12, 70.26, 74.32, 120.69, 121.22, 123.15, 126.05, 127.05, 128.38, 128.54, 128.93, 134.24 134.70, 135.26, 136.22, 136.19, 148.23, 154.45, 155.66, 163.09, 163.11; MS: m/z 441 [M^+]; Anal. calcd. For $C_{24}H_{20}N_6O_6$: C, 59.86; H, 4.38; N, 22.21; Found: C, 56.99; H, 4.22 N, 22.34.

7-(1,3-benzoxazol-2-ylamino)pyrimido[4,5-d]pyrimidin -4(3H)-one 5:

Compound 3b (0.01mol) was added portionwise for 2 h to a mildly refluxing mixture of formic acid (25 mL) and sulfuric acid (1.5 mL). After an additional 30 min, the solution was cooled to 0-5°C and poured onto crushed ice. The resulting precipitate was collected by filtration, washed with water, dried, and crystallized from ethanol to give a fused pyrimidopyrimidinone derivative.

Yield 71%; mp 264-266°C; IR (KBr): 3359, 3186, 3035, 1695, 1602, 1448 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ : 7.12–7.50 (m, 4H, Ar-H), 8.18 (s, 1H, CH fused pyrimidine ring), 7.65 (d, 1H, CH), 5.76 (s, 1H, NH), 9.56 (d, 1H, CON**H**); MS *m/z* 280 $[M]^+$; Anal. calcd. For $C_{13}H_8N_6O_2$: C, 57.72; H, 2.28; N, 29.99; Found: C, 57.64; H, 2.37; N, 30.08.

N-(1,3-benzoxazol-2-yl)-5-chloropyrimido[4,5-d]pyrimidin-2-amine 6:

Compounds 5 (0.05mol), was added to phosphorus oxychloride 30 mL and refluxed on a water bath at 85°C for 5h. The mixture was poured onto ice-cold water. The precipitate formed and was filtered off, dried, and crystallized from ethanol / chloroform.

Yield 68%; mp 214-216°C; IR (KBr): 3360, 3032, 1605, 1440 726 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6):

δ 7.11–7.49 (m, 4H, Ar-H), 8.19 (s, 1H, CH), 7.92 (s, 1H, CH), 5.85 (s, 1H, NH); MS *m/z* 298 [M^+], 300[M^{+2}] ; Anal. calcd. For $C_{13}H_7N_6O$: C, 52.28; H, 2.36; N, 28.14; Found: C, 52.40; H, 2.28; N, 27.99.

General procedure for the synthesis of compounds 7a & 7b:

Compound **6** (0.05mol) and an equivalent molar ratio of the N-hydroxysuccinimide or N-hydroxyphthalimide were dissolve in 20mL DMF and then TEA (2mL) was added drop wise. The reaction mixture was refluxed for 8-10 h. Excess of solvent was distilled and resulting product was filtered, washed with water and recrystallized in ethanol.

1-{[7-(1,3-benzoxazol-2-ylamino)pyrimido[4,5-d]pyrimidin-4-yl]oxy}pyrrolidine-2,5-dione 7a:

Yield 60%; mp 282-284°C; IR (KBr): 3342, 3038, 1695, 1620, 1376, 1435, 1004 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.21–7.62 (m, 4H, Ar-H), 8.35 (s, 1H, CH), 7.90 (s, 1H, CH), 5.81 (s, 1H, NH), 2.52 (m, 4H, CH_2-CH_2): ^{13}C NMR (400 MHz, $CDCl_3$): δ 80.16, 118.08, 120.06, 124.45, 128.34, 128.27, 133.29 133.54, 134.16, 136.22, 137.83, 140.54, 143.92, 146.30, 148.82, 151.65, 154.68, 162.18, 162.19, MS *m/z* 376 [M^+]; Anal. calcd. For $C_{18}H_{12}N_6O_4$: C, 57.45; H, 3.21; N, 22.32; Found: C, 57.40; H, 3.36; N, 22.18.

2-{[7-(1,3-benzoxazol-2-ylamino)pyrimido[4,5-d]pyrimidin-4-yl]oxy}-1H-isoindole-1,3(2H)-dione 7b:

Yield 62%; mp 196-198°C; IR (KBr): 3321, 3030, 1700, 1635, 1358, 1422, 1010 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.11–7.58 (m, 8H, Ar-H), 8.39 (s, 1H, CH), 7.94 (s, 1H, CH), 5.45 (s, 1H, NH), 3.72 (t, 2H, OCH_2), 3.35 (t, 2H, NCH_2); ^{13}C NMR (400 MHz, $CDCl_3$): δ 110.98, 115.56, 118.55, 122.34, 126.35, 130.49 132.22, 134.19, 135.45, 136.88, 138.42, 140.02, 140.54, 142.66, 143.92, 146.30, 148.82, 151.65, 154.68, 155.37, 166.78, 166.82: MS *m/z* 424 [M^+]; Anal. calcd. For $C_{22}H_{12}N_6O_4$: C, 62.27; H, 2.85; N, 19.80; Found: C, 62.40; H, 2.72; N, 20.01.

N^2-(1,3-benzoxazol-2-yl)pyrimido[4,5-d]pyrimidine-2,5-diamine 8:

Compound **3b** (0.01 mol) was dissolved in acetone and formamide (9.8 mL) was added to it. The reaction mixture was refluxed for 5 h. Resultant mixture was cooled and filtered. The filtrate was poured on crushed ice and kept for 3 hrs at room temperature. Solid separated was filtered, dried and recrystallized from glacial acetic acid.

Yield 72%; mp 277-279°C; IR (KBr): 3484, 3365, 3198, 3032, 1605, 1445, 1179 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.45 (s, 2H, NH_2), 8.33 (s, 1H, CH), 7.74 (s, 1H, CH), 5.69(s, NH), 7.16–7.43 (4H, m, Ar-H), 8.48 (1H, s, Ar-H), MS *m/z* 279 [$M]^+$; Anal. calcd. For $C_{13}H_9N_7O$: C, 59.91; H, 3.25; N, 35.11; Found: C, 59.99; H, 3.18; N, 35.17.

2-{2-[7-(Benzooxazol-2-ylamino)-pyrimido[4,5-d]pyrimidin-4-ylamino]-ethoxy}-isoindole-1,3-dione 9:

Compound **8** (0.01 mol) was refluxed in dry acetone (25 mL) containing K_2CO_3 (0.01 mol) as base and phthalimidoxy ethyl bromide **1** (0.01 mol) for 16 h. Excess of solvent was removed under reduced pressure. The separated solid was filtered, washed and recrystallized from ethanol.

Yield 60%; mp 178-180 ^{o}C; IR (KBr): 3392, 3040, 1690, 1635, 1455, 1361, 1162, 1035 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14–7.72 (m, 8H, Ar-H), 5.98 (s, NH), 5.29 (t, 1H, $NHCH_2$), 8.32 (s, 1H, CH), 7.77(s, 1H, CH), 3.88 (t, 2H, OCH_2), 3.45 (t, 2H, NHC**H**$_2$), 5.36 (t, 1H, N**H**CH_2); ^{13}C NMR (400 MHz, $CDCl_3$): δ 47.22, 58.22, 66.41, 69.24, 70.22 118.26, 118.29, 120.31, 120.35, 124.32, 124.37, 128.21, 132.33 134.44, 135.32, 137.48, 142.11, 142.38, 142.68, 142.85, 165.24, MS *m/z* 468 [$M]^+$; Anal. calcd. For $C_{23}H_{16}N_8O_4$: C, 58.97; H, 3.44; N, 23.92; Found: C, 58.88; H, 3.36; N, 23.99.

2-{2-[7-(Benzooxazol-2-ylamino)-4-oxo-4H-pyrimido[4,5-d]pyrimidin-3-yl]-ethoxy}-isoindole-1,3-dione 10:

Compound **5** (0.01mol) and phthalimidoxy ethyl bromide (0.01mol) were dissolved in absolute alcohol (25 mL). Pyridine was added to this solution as a base. The reaction mixture was refluxed for 17 h in around bottom flask. Excess of solvent was distilled of under reduced pressure .The reaction mixture was cooled to room temperature and poured into crushed ice. Products were filtered, washed with cold water, dried and recrystallized from methanol**.**

Yield 63%; mp 165-167 °C; IR (KBr): 3350, 3035, 1689, 1615, 1465, 1374, 1186, 1048 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.16–7.74 (m, 8H, Ar-H), 5.84 (s, NH), 8.32 (s, 1H, CH), 7.76 (s, 1H, CH), 3.85 (t, 2H, OCH_2), 3.35 (t, 2H, NCH_2); ^{13}C NMR (400 MHz, $CDCl_3$): δ 48.31, 59.20, 66.42, 69.32, 71.01 118.11, 118.23, 121.11, 121.36, 123.48, 123.33, 126.08, 132.96 133.29, 135.62, 135.83, 142.82, 142.98, 143.09, 143.22, 169.35, MS *m/z* 469 [$M]^+$; Anal. calcd. For $C_{23}H_{15}N_7O_5$: C, 58.85; H, 3.22; N, 20.89; Found: C, 58.96; H, 3.16; N, 20.95.

Pharmacology:

In-vivo anti-inflammatory activity:

Anti-inflammatory activity was evaluated by the method described by (Winter *et al*., 1962) [37]. Albino rats of either sex weighing 160 – 220 g were divided in 8 groups containing six rats each. Group-1 received 0.5% CMC suspension (control), Group-2 accustomed accepted biologic Diclofenac (50 mg/kg, p.o) respectively. Group 3-8 received test compounds through the same route. Rats were treated with drugs by oral route. Thirty minutes later 0.1 mL of 1% freshly prepared carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the planter aponeurosis of the hind paw. The paw volume is measured by using plethysmograph immediately after injection, and procedure was repeated at 1, 2, 3 and 4 hrs after carrageenan injection. The difference between the initial and subsequent values gave the actual edema volume which was compared with control. The percent anti-inflammatory activity was calculated according to the formula given below-

% Anti-inflammatory activity = $[\ 1 - V_t / V_c\ ] \times 100$

V_t and V_c are paw volumes of edema in tested and control groups, respectively. The activity was monitored at different time interval after administration. It was found that the activity continuously increased with time. The results of i*n-vivo* anti-inflammatory activity is presented in Table **1** and Figure **1**.

Table 1: (*In- vivo* anti-inflammatory activity): Percentage inhibition of test compounds against carrageenan-induced paw edema in albino rats.

Comp.	Dose (mg/kg)	Initial reading	Volume of paw edema after				Inhibition of paw edema after		% Anti-inflammatory activity after	
			1 h	2 h	3 h	4 h	2h	4h	2h	4h
Control	-	3.32 ±0.041	6.51 ±0.024	6.92 ±0.033	6.64 ±0.055	5.49 ±0.018	3.60 ±0.012	2.17 ±0.024	-	-
Diclofenac	50	3.36 ±0.028	4.54 ±0.064	4.65 ±0.042	3.53 ±0.048	3.38 ±0.059	1.29 ±0.021	0.02 ±0.010	64.17	99.07
4a	50	3.39 ±0.031	4.98 ±0.042	5.54 ±0.036	4.81 ±0.069	3.67 ±0.052	2.15 ±0.018	0.28 ±0.012	40.27	87.10
4b	50	3.37 ±0.064	5.12 ±0.029	5.33 ±0.044	4.64 ±0.062	3.58 ±0.019	1.96 ±0.017	0.21 ±0.009	45.55	90.32
7a	50	3.42 ±0.039	4.62 ±0.041	4.95 ±0.064	3.67 ±0.055	3.46 ±0.028	1.53 ±0.014	0.04 ±0.011	**57.50**	98.15
7b	50	3.35 ±0.027	4.54 ±0.044	4.79 ±0.026	3.58 ±0.061	3.48 ±0.034	1.44 ±0.019	0.13 ±0.004	**60.00**	94.01
9	50	3.28 ±0.059	5.04 ±0.037	5.57 ±0.046	4.49 ±0.041	4.01 ±0.067	2.29 ±0.028	0.73 ±0.014	36.38	66.35
10	50	3.32 ±0.033	4.74 ±0.061	5.04 ±0.042	4.53 ±0.091	3.79 ±0.037	1.72 ±0.044	0.47 ±0.018	**52.22**	78.34

Significance levels $P< 0.05$, compared with respective control (ANOVA followed by Dunnett's t- tests). Values are expressed as mean ± SEM, n = 6.

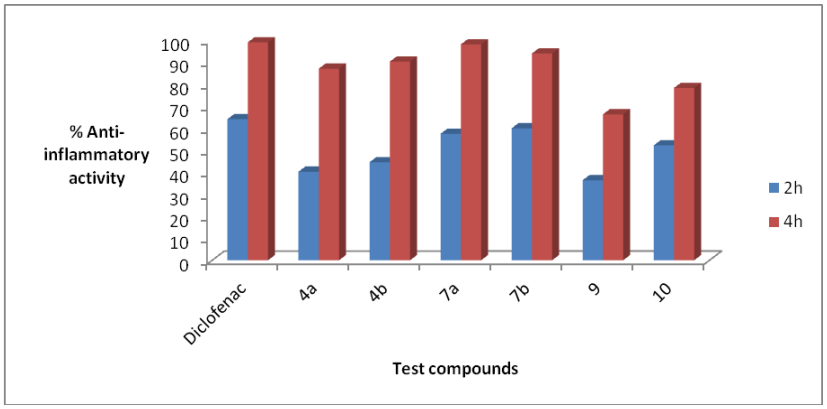


Figure 1: Anti-inflammatory activity of the final compounds.

Statistical analysis:

The statistical analysis of the evaluation of anti-inflammatory activity of the synthesized compounds against the carrageenan induced paw oedema in albino rats were analyzed by using one-way analysis of variance (ANOVA) followed by Dunnett's t test and expressed as mean ± SEM. Differences between the mean of treated animals and control groups were considered significant at $P < 0.05$.

Acknowledgement

The authors are thankful to the Head, Department of Chemistry, M. L. Sukhadia University, Udaipur, India for providing laboratory facilities, the Director, NFDD Rajkot, India for providing spectral and analytical data. One of the authors Mr Prakash Prajapat (NET- SRF) is thankful to UGC, New Delhi, for financial assistance.

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