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Synthesis and biological evaluation of some novel benzoxazine-4-one and quinazolin-4-one derivatives based on anti-inflammatory commercial drugs

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ABSTRACT

Benzoxazine and quinazoline are nitrogen-containing heterocyclic scaffolds found in various biologically active compounds. Due to their diverse biological actions, these heterocyclic rings serve as crucial frameworks for designing medicinal compounds. This study aimed to synthesize and assess *in vivo* anti-inflammatory, analgesic, and low ulcerogenic potential of a few novel benz[*d*][1,3]-oxazine-4-one and quinazolinone derivatives. Benzoxazinones (**3a-e**) were synthesized by cyclizing the carboxylic group (-COOH) of five nonsteroidal anti-inflammatory drugs *viz.,* aceclofenac, ibuprofen, diclofenac, mefenamic acid and ketoprofen (**2a-e**) with anthranilic acid (**1**) using dry phosphorus oxychloride ($POCl_3$) in pyridine. The corresponding quinazolinone derivatives (**5a-e**) were obtained by reacting **3a-e** with isonicotinic acid hydrazide (**4**). Both sets of compounds were evaluated for their anti-inflammatory, analgesic effects, and ulcerogenicity in animal models. Structural characterization was performed using spectral analysis. Among the benzoxazinone derivatives, compound 2-(2-((2,6-dichlorophenyl) amino) benzyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **(3d)** exhibited significant anti-inflammatory activity (62.61% inhibition of rat paw edema) and analgesic activity (62.36% protection in acetic acid-induced writhings) with tolerable gastrointestinal toxicity (2.67 ulcerogenicity index) compared to quinazolinone derivatives. The results of anti-inflammatory and analgesic activities of both the series are comparable with the respective, positive control. Compound **3d**, a benzoxazinone-diclofenac hybrid, emerged as a lead molecule with potent anti-inflammatory, analgesic activities and moderate gastric toxicity showcasing the promising potential for further development.

Keywords: Anti-inflammatory, analgesic, benzoxazinone, heterocyclic, quinazolinone.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) represent a cornerstone in the treatment and management of various inflammatory conditions, addressing both pain and fever. However, their long-term use has been associated with gastrointestinal (GI) toxicity [1]. This issue arises primarily because most conventional NSAIDs clinically utilized inhibit one isoform of the cyclooxygenase (COX) enzyme namely the constitutive COX-1 enzyme. This particular enzyme plays a crucial role in safeguarding the gastric mucosa by producing prostaglandin I_2 (PGI_2) and PGE_1 [2]. Consequently, the inhibition of COX-1 by NSAIDs leads to a decrease in the levels of these protective prostaglandins, resulting in GI side effects ranging from hemorrhage to peptic ulcers [3]. Additionally, the presence of a free carboxylic group in traditional NSAIDs can exacerbate GI toxicity by inducing a local corrosive effect, further compromising the integrity of the GI mucosa. Therefore, research efforts have been concentrated on the development of selective COX-2 inhibitors devoid of GI toxicity. However, initial first-generation COX-2 inhibitors, such as rofecoxib and valdecoxib, approved by the Food and Drug Administration (FDA), were withdrawn from the market due to elevated risks of cardiovascular complications including heart attack and stroke [4, 5]. Consequently, safer alternatives were sought, leading to the

development of second-generation COX-2 inhibitors such as celecoxib and other Coxibs, which clinicians now prefer over traditional NSAIDs for managing inflammatory conditions owing to their reduced toxicity [6]. Furthermore, one of the strategies to mitigate GD toxicity and enhance the therapeutic efficacy of NSAIDs has involved modifying the free-COOH group through cyclization to a heterocyclic ring [7]. In our previous investigations, felbinac, an active metabolite of fenbufen, underwent rational modification wherein its free carboxylic group was substituted with biologically active heterocyclics such as triazole, oxadiazole, and thiadiazole. Among the prepared derivatives, an oxadiazole derivative exhibited significantly diminished ulcerogenic effects while demonstrating superior analgesic and anti-inflammatory activity compared to felbinac. Additionally, several derivatives exhibited enhanced selectivity in COX-2 inhibition when compared to felbinac [8]. Many similar studies have also indicated that the inclusion of a heterocyclic moiety containing nitrogen atoms may ameliorate gastric tolerability while broadening the anti-inflammatory spectrum of compounds [9, 10].

In recent years, benz-fused ring systems such as benzoxazinone and quinazolinone, the nitrogen-containing heterocyclic systems, have garnered significant interest among chemists due to their broad array of biological activities [11, 12]. Some of these derivatives have been noted for their selective inhibition of COX-2 enzymes alongside exhibiting improved GI profile potentially attributed to the absence of a free-COOH group within their structures [13]. Sachin *et al*., reported benzothiazole-quinazolinone hybrids to be weak anti-inflammatory agents yet they demonstrated enhanced selectivity as COX-2 inhibitors with a favorable GI safety profile [14]. Benzoxazinone derivatives have been documented to exhibit a plethora of activities including anti-inflammatory, analgesic, anti-tubercular, anti-microbial, antipsychotic, and central nervous system (CNS) depressant properties, among others [15 - 18]. Khalaj *et al.,* in 2002 showcased that 4H-2,3-dihydro-1,3-benzoxazine, a cyclic acetal-like derivative of salicylamide, was more potent than positive control aspirin in exhibiting *in vivo* antinociceptive and anti-inflammatory activities without causing gastric mucosal injury. The tested compound at a dose of 1 mmol/kg showed a reduction of 99% and 100% formalin-induced rat paw edema after 2 h and 24 h respectively in comparison to aspirin (63% and 73% reduction) [19]. The chemical structures of some of the pharmaceutical drugs containing benzoxazinone moiety are presented in Fig. 1.

Quinazolinone, an example of a benz-fused heterocyclic ring system, serves as a nitrogen analogue of benzoxazinone moiety and is the oxidized form of quinazoline. It features prominently in many naturally occurring bioactive compounds such as alkaloids and vitamins [20 - 22]. It has been used as a crucial building

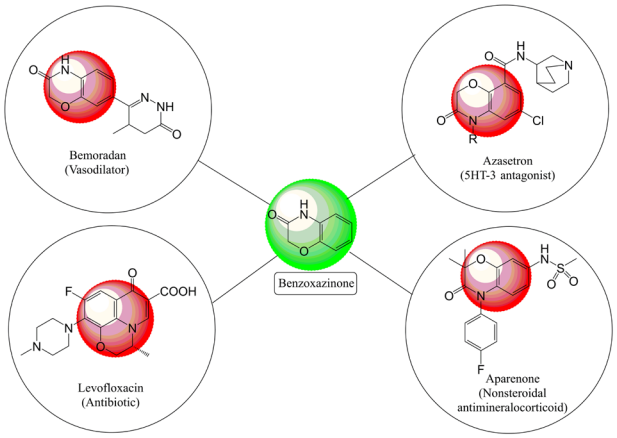


Fig. 1. Chemical structures of benzoxazinone-containing drugs.

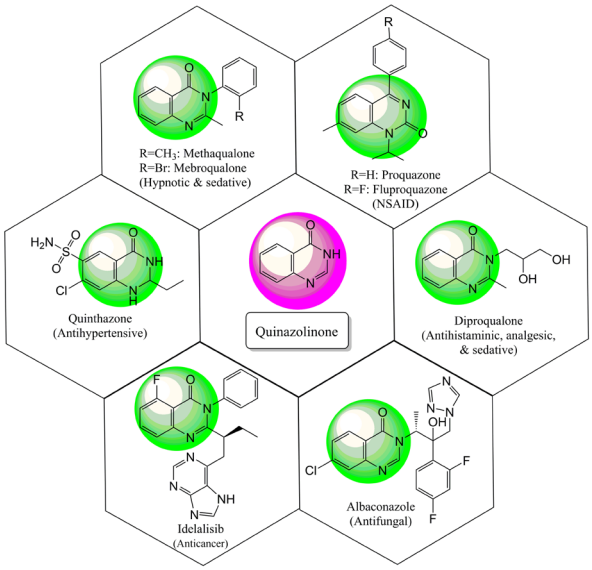


Fig. 2. Chemical structures of quinazolinone-containing drugs.

block for the synthesis of useful medicinal products. Quinazolinone-containing compounds have been demonstrated to possess diverse pharmacological activities including antimicrobial, anticonvulsant, anticancer, antidiabetic, anti-hypertensive, CNS depressant, analgesic, and anti-inflammatory properties [23 - 26]. El-Hashash *et al.,* in 2016 reported the anti-inflammatory activity of some novel quinazolinone derivatives. The synthesized compounds exhibited promising anti-inflammatory and antimicrobial activities [27]. The chemical structures of some quinazolinone-containing drugs are illustrated in Fig. 2.

In light of these observations, we have incorporated benzoxazinone and quinazolinone scaffolds into the core structures of five clinically used NSAIDs namely aceclofenac, ibuprofen, diclofenac, mefenamic acid, and ketoprofen [28] in hope of obtaining hybrid compounds possessing potent anti-inflammatory activity. We designed, synthesized, and evaluated novel

derivatives of 4*H*-benz[*d*][1,3]-oxazine-4-one and their nitrogen analogs for their *in vivo* anti-inflammatory and analgesic effects while also evaluating their potential for inducing ulcerogenicity.

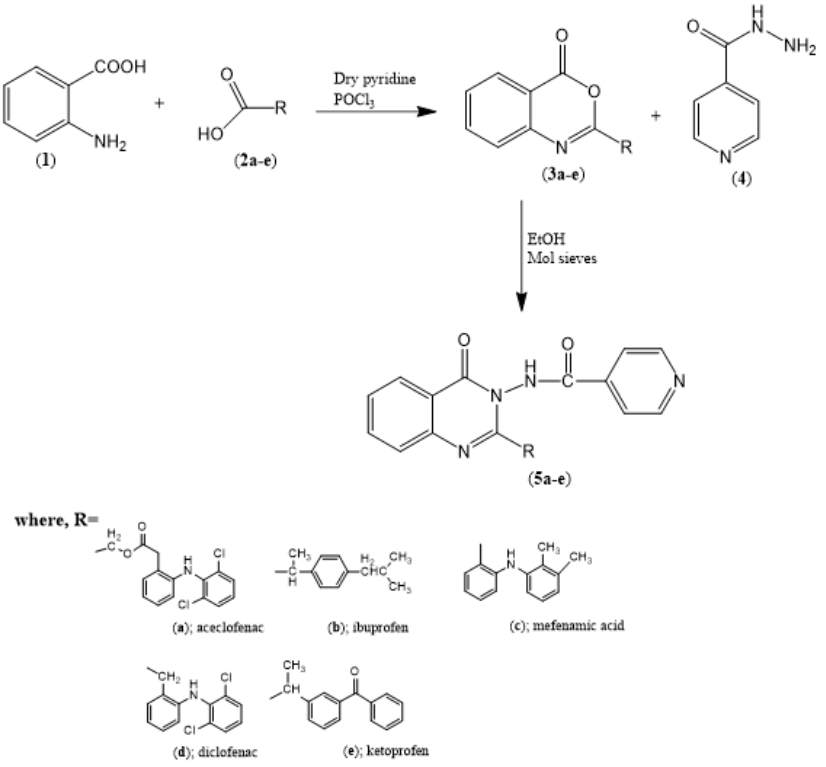
EXPERIMENTAL

All chemicals and solvents used in the current study were purchased from various chemical units like E. Merck (India) Ltd., S.D. Fine and C.D.H. Pure samples of NSAIDs were received as generous gifts from Arbro Pharmaceuticals, New Delhi. Progress of the reaction and purity of the synthesized compounds were checked on thin layer chromatography (TLC) plates using solvent systems benzene : acetone (9:1). The Iodine chamber and UV lamp were used for the visualization of TLC spots. All the melting points were determined in an open glass capillary using a Kjeldahl flask containing liquid paraffin and are uncorrected. The structures of the synthesized compounds were established based on IR, ^{1}H-NMR, and Mass spectral data results. IR spectra were recorded in KBr on FT-IR (Jasco, 410). The Proton Magnetic Resonance Spectra (^{1}H-NMR) was recorded in Dimethyl Sulfoxide (DMSO) on an NMR Spectrometer (Bruker, DRX-300), using tetra-methyl silane (TMS) as an internal reference. The Mass spectra were recorded on liquid chromatography coupled with a mass spectrometer (LCMS/MS; PerkinElmer and LABINDIA, Applied Biosystem, API 3000).

General procedure for the synthesis of benzoxazine derivatives (3a-e): The reactants: (i) anthranilic acid (4.38 mmol; 0.6 g) and (ii) appropriate NSAID (4.34 mmol) were dissolved separately in dry pyridine (5 mL) and the two solutions were mixed under ice-cold conditions followed by dropwise addition of $POCl_3$ (0.5 mL) and stirring for 8 h while maintaining the temperature below 5 °C. The reaction was then quenched by adding ice-cold water. A solid mass that separated was filtered, washed with water, and crystallized from methanol solvent.

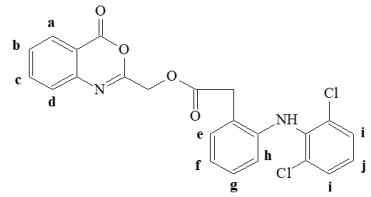
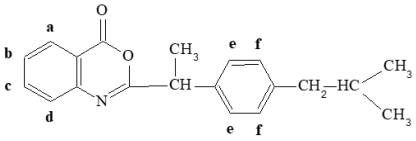
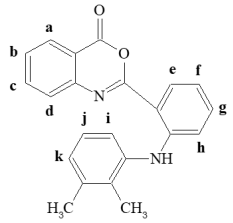
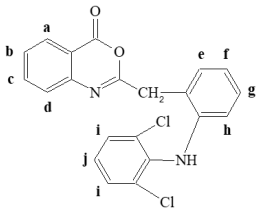
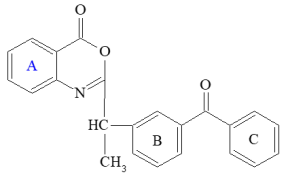
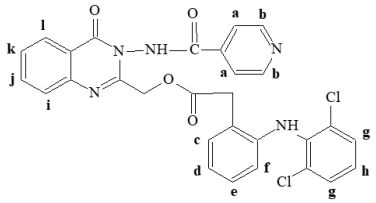
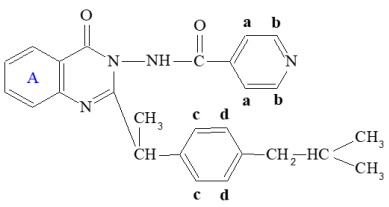
Synthesis of (4-oxo-4H-benzo[d][1,3] oxazin-2-yl) methyl 2-(3-((2,6-dichlorophenyl) amino) phenyl) acetate (3a): Aceclofenac (**2a**; 4.34 mmol; 1.537 g) and anthranilic acid (**1**; 4.38 mmol; 0.6 g) were condensed to obtain **3a**. The compound was crystallized from methanol to obtain a TLC pure light brown crystalline powder. Melting point: 166 - 168 °C; Yield: 79%; Partition coefficient: 6.31; R_f value: 0.73 (benzene : acetone; 9 : 1). Spectral data in Table 1.

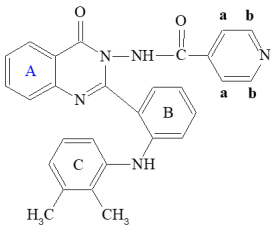
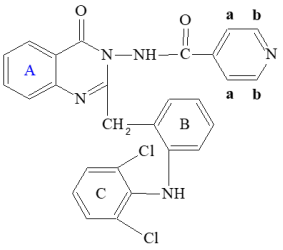
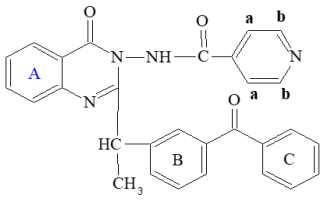
Synthesis of 2-(1-(4-isobutylphenyl) ethyl)-4H-benzo[d][1,3]oxazin-4-one (3b): Ibuprofen (**2b**; 4.34 mmol; 0.895 g) and anthranilic acid (**1**; 4.38 mmol; 0.6 g) were condensed to obtain **3b**. The compound was crystallized from methanol to obtain a TLC pure light brown crystalline powder. Melting point: 107-109 °C; Yield: 82%; Partition coefficient: 5.82; R_f value: 0.72 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.



Scheme 1. Protocol for the synthesis of benzoxazinone and quinazolinone derivatives.

Table 1. Spectral data of synthesized benzoxazine and quinazolinone derivatives

Comp.	Chemical structure	Spectral data
3a		**IR spectral data (KBr/v_{max} cm^{-1}):** 3090 (Ar C-H), 1681 (C=O, ketone), 1516 (N-H, aromatic amine), 1338 (C-N, aromatic amine), 1096 (C-Cl).; **^{1}H NMR spectral data (δ in ppm):** 4.06 (s, 2H, $COCH_2$), 4.74 (s, 2H, CH_2O), 6.21 (s, 1H, NH), 6.83-6.95 (m, 2H, $H_{h,l}$), 7.03-7.19 (m, 4H, $H_{b,c,f,g}$), 7.28 (d, 1H, H_a), 7.39 (d, 1H, H_d), 7.79 (d, 1H, H_e), 8.52-8.57 (m, 2H, 2xH_i).
3b		**IR spectral data (KBr/v_{max} cm^{-1}):** 3023 (Ar C-H), 1678 (C=O, ketone); **^{1}H NMR spectral data (δ in ppm):** 0.82 (d, 6H, 2xCH_3), 1.44 (d, 3H, $CHCH_3$), 1.77 (m, 1H, CH_2CH), 2.39 (d, 2H, CH_2CH), 3.01 (q, 1H, $CHCH_3$), 6.97-7.28 (m, 5H, 2x$H_{e,f}$+H_c), 7.52 (t, 1H, H_b), 7.93 (d, 1H, H_d), 8.51 (d, 1H, H_a); **Mass spectral data (m/z):** 307 (M^+), 292, 277.
3c		**IR spectral data (KBr/v_{max} cm^{-1}):** 3069 (Ar C-H), 1676 (C=O, ketone), 1514 (N-H, aromatic amine), 1305 (C-N, aromatic amine); **^{1}H NMR spectral data (δ in ppm):** 2.09 (s, 3H, CH_3 meta to NH), 2.28 (s, 3H, CH_3), 6.24 (s, 1H, NH), 6.66 (t, 1H, H_f), 6.81 (d, 1H, H_h), 7.02 (d, 2H, $H_{i,k}$), 7.11-7.18 (m, 3H, $H_{d,e,g}$), 7.28 (t, 2H, $H_{c,j}$), 7.69 (dd, 1H, H_b), 7.86 (d, 1H, H_a).
3d		**IR spectral data (KBr/v_{max} cm^{-1}):** 3080 (Ar C-H), 1670 (C=O, ketone), 1516 (N-H, aromatic amine), 1299 (C-N, aromatic amine), 1089 (C-Cl); **^{1}H NMR spectral data (δ in ppm):** 3.89 (s, 2H, CH_2), 6.27 (s, 1H, NH), 7.09 (d, 1H, H_h), 7.21 (d, 1H, H_d), 7.33 (dd, 2H, 2xH_i), 7.50-7.63 (m, 4H, $H_{b,c,f,g}$), 7.76 (d, 1H, H_a), 7.88 (t, 1H, H_j), 7.98 (d, 1H, H_e).
3e		**IR spectral data (KBr/v_{max} cm^{-1}):** 3080 (Ar C-H), 1673 (C=O, aromatic ketone); **^{1}H NMR spectral data (δ in ppm):** 1.41 (d, 3H, CH_3), 3.38 (q, 1H, CH), 6.92-7.08 (m, 4H, ring B), 7.16-7.28 (m, 5H, ring C), 7.43-7.55 (m, 4H, ring A).
5a		**IR spectral data (KBr/v_{max} cm^{-1}):** 3222 (N-H, amide), 3094 (ArC-H), 1666 (C=O, ketone), 1638 (C=O, amide), 1515 (N-H, aromatic amine), 1328 (C-N, aromatic amine), 1279 (C-O, ester), 1090 (C-Cl), 755 (C-N, amide); **^{1}H NMR spectral data (δ in ppm):** 3.55 (s, 2H, $COCH_2$), 3.64 (s, 2H, CH_2O), 6.21 (s, 1H, NH), 6.29 (d, 2H, 2xH_g), 6.86 (t, 2H, $H_{e,j}$), 7.05 (t, 2H, $H_{d,k}$), 7.17-7.23 (m, 5H, $H_{c,f,h,i,l}$), 7.51 (m, 4H, 2x$H_{a,b}$), 10.60 (s, 1H, NHCO).
5b		**IR spectral data (KBr/v_{max} cm^{-1}):** 3110 (N-H, amide), 3092 (Ar-C-H), 1678 (C=O, ketone), 1640 (C=O, amide), 751 (C-N, amide); **^{1}H NMR spectral data (δ in ppm):** 1.10 (d, 6H, 2xCH_3), 1.32 (d, 3H, $CHCH_3$), 2.09 (m, 1H, CH_2CH), 2.62 (d, 2H, CH_2CH), 3.02 (q, 1H, $CHCH_3$), 7.46-7.57 (m, 4H, 2x$H_{c,d}$), 7.76-7.80 (m, 4H, ring A), 7.90-7.94 (m, 4H, 2x$H_{a,b}$), 8.67 (s, 1H, NH); **Mass spectral data (m/z):** 424 (M^+), 425 (M-1), 405, 390, 376.

5c		**IR spectral data (KBr/v_{max} cm^{-1}):** 3068 (Ar C-H), 1675 (C=O, ketone), 1517 (N-H, aromatic amine), 1333 (C-N, aromatic amine); **^{1}H NMR spectral data (δ in ppm):** 2.10 (s, 3H, CH_3 meta to NH), 2.28 (s, 3H, CH_3), 6.23 (s, 1H, NH), 6.68-6.76 (m, 4H, ring A), 7.01-7.12 (m, 4H, ring B), 7.26-7.32 (m, 3H, ring C), 7.87-7.91 (m, 4H, 2x$H_{a,b}$), 9.66 (s, 1H, NHCO).
5d		**IR spectral data (KBr/v_{max} cm^{-1}):** 3231 (N-H, amide), 3043 (Ar C-H), 1668 (C=O, ketone), 1640 (C=O, amide), 1516 (N-H, aromatic amine), 1330 (C-N, aromatic amine), 1094 (C-Cl), 781 (C-N, amide); **^{1}H NMR spectral data (δ in ppm):** 3.88 (s, 2H, CH_2), 6.22 (s, 1H, NH), 6.70-6.88 (m, 4H, ring A), 6.90-7.18 (m, 3H, ring C), 7.20-7.27 (m, 4H, ring B), 7.57-7.76 (m, 4H, 2x$H_{a,b}$), 8.82 (s, 1H, NHCO); **Mass spectral data (m/z):** 516 (M^+), 437, 411, 379, 278, 214, 149, 121.
5e		**IR spectral data (KBr/v_{max} cm^{-1}):** 3244 (N-H, amide), 3083 (Ar C-H), 1676 (C=O, ketone), 1639 (C=O, amide), 789 (C-N, amide); **^{1}H NMR spectral data (δ in ppm):** 1.38 (d, 3H, CH_3), 3.38 (q, 1H, CH), 6.95-7.09 (m, 4H, ring B), 7.11-7.26 (m, 5H, ring C), 7.41-7.53 (m, 4H, ring A), 7.98-8.22 (m, 4H, 2x$H_{a,b}$), 8.97 (s, 1H, NHCO).

Synthesis of 2-(3-((2,3-dimethylphenyl) amino) phenyl)-4H-benzo[d][1,3]oxazin-4-one (3c): Mefenamic acid (**2c**; 4.34 mmol; 1.047 g) and anthranilic acid (**1**; 4.38 mmol; 0.6 g) were condensed to obtain **3c**. The compound was crystallized from methanol to obtain a TLC pure mustard-colored crystalline powder. Melting point: 169 - 171 °C; Yield: 81%; Partition coefficient: 6.01; R_f value: 0.75 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.

Synthesis of 2-(2-((2,6-dichlorophenyl) amino) benzyl)-4H-benzo[d][1,3]oxazin-4-one (3d): Diclofenac (**2d**; 4.34 mmol; 1.285 g) and anthranilic acid (**1**; 4.38 mmol; 0.6 g) were condensed to obtain **3d**. The compound was crystallized from methanol to obtain a TLC pure orange crystalline powder. Melting point: 117 - 119 °C; Yield: 88%; Partition coefficient: 6.50; R_f value: 0.82 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.

Synthesis of 2-(1-(2-benzoylphenyl) ethyl)-4H-benzo[d][1,3]oxazin-4-one (3e): Ketoprofen (**2e**; 4.34 mmol; 1.104 g) and anthranilic acid (**1**; 4.38 mmol; 0.6 g) were condensed to obtain **3e**. The compound was crystallized from methanol to obtain a TLC pure light brown crystalline powder. Melting point: 109 - 111 °C; Yield: 69%; Partition coefficient: 5.76; R_f value: 0.67 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.

General procedure for the synthesis of quinazolinone derivatives (5a-e): The reactants: (i) Isoniazid (**4**) and (ii) respective benzoxazinones **(3a-e)** were dissolved in absolute ethanol (10 mL) and were refluxed (24 - 48 h) in the presence of molecular sieves. The reaction was then decomposed by evaporation of excess ethanol and the addition of ice-cold water. A solid mass that separated was filtered, washed with water, and crystallized using a suitable solvent.

Synthesis of (3-(isonicotinamido)-4-oxo-3,4-dihydroquinazolin-2-yl) methyl 2-(3-((2,6-dichlorophenyl) amino) phenyl) acetate (5a): A mixture of **3a (**4.0 mmol; 1.821 g) and Isoniazid (**4**; 4.0 mmol; 0.548 g) were reacted as per the general procedure to obtain **5a**. The compound was crystallized from methanol to obtain a TLC pure creamy crystalline powder. Melting point: 192 - 194 °C; Yield: 65%; Partition coefficient: 5.39; R_f value: 0.54 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.

Synthesis of N-(2-(1-(4-isobutylphenyl) ethyl)-4-oxoquinazolin-3(4H)-yl) isonicotinamide (5b): A mixture of **3b** (4.0 mmol; 1.230 g) and Isoniazid (**4**; 4.0 mmol; 0.548 g) were reacted as per the general procedure to obtain **5b**. The compound was crystallized from methanol to obtain a TLC pure mustard colored crystalline powder. Melting point: 123 - 126 °C; Yield: 71%; Partition coefficient: 4.90; R_f value: 0.55 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.

Synthesis of N-(2-(3-((2,3-dimethylphenyl) amino) phenyl)-4-oxoquinazolin-3(4H)-yl) isonicotinamide (5c): A mixture of **3c** (4.0 mmol; 1.370 g) and Isoniazid (**4**; 4.0 mmol; 0.548 g) were reacted as per the general procedure to obtain **5c**. The compound was crystallized from methanol to obtain a TLC pure brown crystalline powder. Melting point: 189 - 191 °C; Yield: 69%; Partition coefficient: 5.09; R_f value: 0.56 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.

Synthesis of N-(2-(2-((2,6-dichlorophenyl) amino) benzyl)-4-oxoquinazolin-3(4H)-yl) isonicotinamide (5d): A mixture of **3d** (4.0 mmol; 1.60 g) and Isoniazid (**4**; 4.0 mmol; 0.548 g) were reacted as per the general procedure to obtain **5d**. The compound was crystallized from methanol to obtain a TLC pure pale-yellow crystalline powder. Melting point: 229 - 231°C; Yield: 78%; Partition coefficient: 5.58; R_f value: 0.60 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.

Synthesis of N-(2-(1-(2-benzoylphenyl) ethyl)-4-oxoquinazolin-3(4H)-yl) isonicotinamide (5e):
A mixture of **3e** (4.0 mmol; 1.422 g) and Isoniazid (**4**; 4.0 mmol; 0.548 g) were reacted as per the general procedure to obtain **5e**. The compound was crystallized from methanol to obtain a TLC mustard-colored crystalline powder. Melting point: 157 - 159°C; Yield: 56%; Partition coefficient: 4.84; R_f value: 0.62 (benzene : acetone; 9 : 1). Spectral data is given in Table 1.

Pharmacological evaluation: Both series of benzoxazinone and quinazolinones were screened for anti-inflammatory, analgesic, and GI toxicity studies as per the reported methods [29, 30]. Before the animal experimental studies, ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard University. All the experiments were performed as per the recommended protocol of CPCSEA.

Anti-inflammatory activity by carrageenan method: Anti-inflammatory activity was assessed in Wistar Albino rats of either sex (150-200 g) using a digital Plethysmometer (Ugo Basile, 7140). Animals were divided into groups having six rats in each group. These were labelled as; group 1 (carrageenan control), group 2-6 (standard NSAIDs), and group 7-15 (test compounds). 1% w/v suspension of carrageenan prepared in 0.5% sodium CMC was used to induce inflammation. Standard NSAIDs in a dose of 20 mg/kg prepared in CMC were administered orally. The doses of all synthesized compounds were calculated molecularly equivalent to the dose of 20 mg/kg of the parent drugs and administered so as suspension. Reduction in inflammation (paw edema volume or swelling) was calculated following the previously reported method [30].

Analgesic activity by acetic acid-induced Writhing method: Analgesic activity was evaluated by a previously reported method using healthy albino mice of either sex (25-30 g) [31]. 1% v/v acetic acid (writhing inducing agent) in a dose of 1 mL/100 g body weight was injected intraperitoneally (IP) to all groups. Standard NSAIDs and test compounds were administered orally in a dose of 20 mg/kg body weight of animals. % protection in terms of a decrease in a number of acetic acid-induced writhings was calculated to assess the analgesic activity.

Acute ulcerogenic activity: Acute GI toxicity was determined on healthy Wistar rats of either sex (150-200 g) following the standard reported method [30]. A single dose of standard NSAIDs and test compounds at the doses of 25, 50, and 100 mg/kg of body weight in Sodium CMC were administered orally to fasting rats for 8 h and then sacrificed after 17 h of dosing. The mucosal damage in the dissected and opened stomach was measured to evaluate and compare the ulcerogenic activity (severity index) of drugs and test compounds.

RESULTS AND DISCUSSION

Chemistry: Five novel benzoxazinone derivatives (**3a-e**) were synthesized by condensation of clinically used anti-inflammatory drugs (aceclofenac, ibuprofen, mefenamic acid, diclofenac, and ketoprofen) with anthranilic acid in the presence of $POCl_3$ in dry pyridine as per Scheme 1. The compounds were purified by recrystallization in methanol and were obtained in good to excellent yield (69 - 88%). The chemical structures of benzoxazinone derivatives were established based on IR, NMR, and mass spectral data (Table 1). IR spectra of **3a-e** showed characteristic peaks for C=O and C-N groups. All the protons of synthesized title compounds were accounted for in the ^{1}H NMR spectra. ^{1}H NMR spectrum of all benzoxazinone derivatives showed an additional signal for four protons in the aromatic region which was attributed to the phenyl ring of benzoxazinone confirming their formation from respective NSAID.

A total of 5 novel quinazolinone derivatives **(5a-e)** were prepared from benzoxazinones **(3a-e)** in a single step by condensation with isoniazid (**4**) in the presence of molecular sieves as per protocol outlined in Scheme 1. These compounds after crystallization gave a single stop on TLC indicating them to be pure enough for further characterization and biological testing. However, these were obtained in a lower % yield (56 - 78%) than benzoxazinone derivatives. In IR, a characteristic amide (C=O-NH) peak of isonicotinamide was seen for all quinazolinone derivatives in addition to the C=O and C-N peaks. ^{1}H NMR and mass spectral data are also in agreement with their formation.

Pharmacological evaluation: Anti-inflammatory activity (% inhibition of inflammation) of the standard NSAIDs and synthesized compounds were evaluated after 2 h and 4 h of administration to rats. In general, the parent drug showed better anti-inflammatory activity than the benzoxazinone and quinazolinone compounds after both 2 h and 4 h (Table 2). All the tested compounds exhibited a higher % inhibition of inflammation at 4 h. Diclofenac was observed to be the most potent inflammatory drug among all the five NSAIDs with a % inhibition of 62.48 and 68.20 after 2 h and 4 h, respectively. The results of anti-inflammatory activity are presented in Table 2.

It can be seen that benzoxazinone derivatives (**3a-e;** 37.52 - 43.33 at 2 h and 39.93 - 46.19 at 4 h) inhibited carrageenan-induced swelling more strongly than the quinazolinone derivatives (**5a-e**; 35.41 - 41.2 at 2 h and 38.25 - 42.9 at 4 h) suggesting them to be more potent

Table 2. Percentage of swelling and inhibition caused against carrageenan-induced rat paw edema at the 2nd and 4th hour of drug administration.

Treatment	% Inhibition	
	2nd hr	4th hr
Aceclofenac	49.13	52.96
3a	43.33	46.19
5a	41.20	42.90
Ibuprofen	58.71	63.62
3b	55.42	60.24
5b	51.41	58.12
Mefenamic acid	42.07	45.69
3c	37.52	39.93
5c	35.41	38.25
Diclofenac	62.48	68.20
3d	60.00	62.61
5d	56.31	59.11
Ketoprofen	55.10	60.41
3e	51.20	54.00
5e	50.22	52.32

anti-inflammatory agents. Diclofenac-benzoxazinone-conjugate (**3d**) exhibited the best anti-inflammatory activity among the synthesized compounds (60.00 and 62.61% at 2 h and 4 h, respectively) while in the same series, mefenamic acid derivative (**3c**) showed the least % inhibition of swelling. It could be suggested that replacing the oxygen atom of benzoxazinone with a nitrogen atom to obtain a respectivre quinazolinone derivative with an additional nicotinamide moiety does not improve anti-inflammatory activity. Mymoona *et al*., synthesized and evaluated the anti-inflammatory activity of 1-(3-Phenyl-3,4-dihydro-2H-benzo[*e*][1,3] oxazin-6-yl)-ethanone derivatives. Similar to the results of the current study, the benzoxazinone derivatives exhibited mild to excellent anti-inflammatory activity (25 - 83.3%). Interestingly, only one compound was found to be more potent than the reference drug, indomethacin [32]. El-Hashash *et al*., in 2016 described the synthesis of benzoxazinone and quinazolinone derivatives. The benzoxazine namely *N*-(1-(6,8-Dibromo-4-oxo-4*H*-benzo[*d*][1,3]-oxazine-2-yl)-2-phenylvinyl) benzamide produced comparable anti-inflammatory activity (87.12% inhibition of rat paw edema) concerning positive control indomethacin (94.54% edema inhibition) at 4 h. On the other hand, quinazoline derivatives showed a % reduction in edema from 70.45 - 91.66% after 4 h indicating that some of them exhibited more potent activity than benzoxazine derivatives. The difference in activity could be due to the difference in structure and substitution on the two rings [27]. Zayed and Hassan in 2014 showed that 6,8-diiodo-2-methyl-3-substituted-quinazolin-4(*3H*)-one bearing sulfonamide derivatives at a dose of 50 mg/kg possess moderate to high anti-inflammatory activity (27.47 - 62.83%) compared to Ibuprofen (69.91%) [33].

The results of analgesic activity as illustrated in Fig. 3 indicate that compounds of both the series showed enhanced analgesic activity as compared to their respective parent drugs. The maximum analgesic activity was exhibited by **3a** with 67.26% in comparison to the aceclofenac (65.14%). Diclofenac-benzoxazine derivative (**3d**) also exhibited comparable analgesic activity (62.36%). Surprisingly ibuprofen (**2b**) and ketoprofen (**2e**) parent drugs and their derivatives (**3b, 3e, 5b, and 5e**) displayed the lowest analgesic activity. The benzoxazinone derivatives were found to be potent analgesic agents with respect to quinazolinone derivatives.

A study in 2019 identified six new 1,4-dihydroquinazolin-3(*2H*)-yl benzamide derivatives exhibiting potent *in vitro* COX-2 inhibitory activity (IC_{50} 0.04 - 0.07 µmol) and two of these compounds produced 4 - 21 times higher analgesic effect than diclofenac sodium and indomethacin [13]. Results of a similar study on benzoxazinone derivatives showed them to possess good to excellent analgesic activity against acetic acid-induced writhings (53.43 - 65.35% protection) but their efficacy was lower than the standard indomethacin (66.25%) [32].

The results of GI toxicity (severity index) presented in Table 3 indicated that the parent drugs (standard NSAIDs) were highly ulcerogenic and the severity index increased with an increase in dose from 25 mg/kg to 100 mg/kg. Among the standard NSAIDs, aceclofenac, a prodrug of diclofenac produced the least GI toxicity (0.84 at 25 mg/kg; 1.08 at 50 mg/kg, and 1.50 at 100 mg/kg) while diclofenac caused the most GI damage (1.42, 2.33 and 2.66 at 25, 50 and 100 mg/kg, respectively).

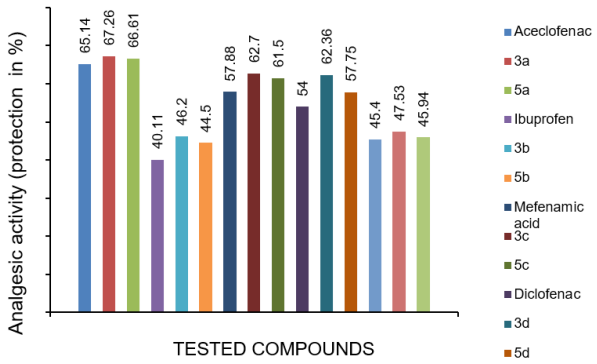


Fig. 3. Percentage protection in acetic acid-induced writhings by standard NSAIDs and their derivatives.

Based on the severity index at three doses of 25, 50, and 100 mg/kg, benzoxazinone (0.92 - 1.50; 1.17 - 2.50 and 1.83 - 2.67) appeared to be safer than quinazolinone derivatives (1.1 - 1.60; 1.3 - 2.75 and 1.96 - 2.84) and their GI toxicity is nearly equal to the parent drugs (0.84 - 142; 1.08 - 2.33 and 1.50 - 2.66). The ulcerogenicity

Table 3. Ulcerogenic activity (severity index) of the synthesized compounds and their respective parent drugs

Treatment	Severity index ± SEM		
	25 mg/kg	50 mg/kg	100 mg/kg
Aceclofenac	0.84 ± 0.16	1.08 ± 0.13	1.50 ± 0.224
3a	0.92 ± 0.08	1.17 ± 0.10	1.83 ± 0.30
5a	1.20 ± 0.09	1.30 ± 0.12	1.96 ± 0.35
Ibuprofen	1.33 ± 0.28	2.00 ± 0.26	2.50 ± 0.22
3b	1.42 ± 0.08	2.10 ± 0.20	2.50 ± 0.22
5b	1.50 ± 0.07	2.83 ± 0.28	2.70 ± 0.26
Mefenamic acid	0.92 ± 0.15	1.33 ± 0.16	1.92 ± 0.23
3c	1.00 ± 0.30	1.42 ± 0.20*	2.16 ± 0.28
5c	1.10 ± 0.20	1.45 ± 0.31*	2.40 ± 0.22
Diclofenac	1.42 ± 0.20	2.33 ± 0.21	2.66 ± 0.21
3d	1.50 ± 0.18	2.50 ± 0.22	2.67 ± 0.21
5d	1.60 ± 0.19	2.75 ± 0.21	2.84 ± 0.28
Ketoprofen	1.17 ± 0.17	1.91 ± 0.23	2.33 ± 0.21
3e	1.25 ± 0.21	2.08 ± 0.32	2.50 ± 0.22
5e	1.31 ± 0.24	2.20 ± 0.27	2.60 ± 0.24

*p value < 0.01 as compared to control

index of benzoxazine derivative (**3a-e**) of each NSAID is lower than the respective quinazolinone derivative (**5a-e**). The benzoxazine derivative of aceclofenac (**3a**) showed a severity index of 1.83 at 100 mg/kg dose which makes it less GI toxic than ibuprofen (2.50), mefenamic acid (1.92), diclofenac (2.66) and ketoprofen (2.33) at the same dose. The severity index of the synthesized compounds (least toxic to the most toxic) was noted in the following order (3a<5a<3c<5c <3b~3e<5e<3d<5b<5d). The title compounds treated groups showed somewhat intact mucosal layers very similar to the standard drugs.

CONCLUSIONS

Two libraries of benzoxazinone and quinazolinone compounds were synthesized using five clinically used anti-inflammatory drugs. Benzoxazinone derivatives were synthesized via a one-pot synthetic method, wherein NSAIDs were reacted with anthranilic acid in the presence of dry phosphorus oxychloride in pyridine. Quinazolinones were obtained by reacting benzoxazinone with isoniazid in the presence of molecular sieves through a single step.

The synthesized compounds exhibited enhanced analgesic activities compared to the parent drugs, while their GI toxicity and anti-inflammatory activity were comparable to the respective NSAIDs. Notably, benzoxazinone derivatives displayed superior analgesic and anti-inflammatory activity and better tolerability than the quinazolinone derivatives. Among them, compound number **3d,** a benzoxazinone derivative of diclofenac, emerged as a promising lead molecule with potent anti-inflammatory and analgesic activities coupled with reduced GI toxicity.

Further modifications are warranted on benzoxazinone derivatives to develop potent and efficacious agents for anti-inflammatory therapy.

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