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Synthesis and Docking Studies of Oxazine Derivatives as Anti-inflammatory and Antioxidant Agents

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ABSTRACT

A new series of N-(4-{4-(substituted phenyl)-2-[(4-fluro-benzylidene)-amino]-6H-[1,3] oxazine-6-yl}-phenyl)-isonicotiamide were synthesized by reaction of p-amino acetophenone with isonicotinyl chloride to obtain the secondary amides. ClaisenSchmidth condensation reaction with substituted aldehydes led to the formation of corresponding chalcones. These chalcones were cyclized with urea to form oxazine which further reacted with p-fluorobenzaldehyde to form the corresponding Schiff's bases. All the synthesized compounds were characterized by IR and ^{1}HNMR spectral data, mass spectra and elemental analysis. The synthesized oxazine and Schiff's bases of oxazine were docked against protein cyclooxygensase target especially selective COX-2. The invitro anti-inflammatory and antioxidant studies of the synthesized compounds were carried out and the results of the docking scores were in correlation with their obtained biological activity.

Keywords: Oxazine, Schiff's base, Antiinflammatory, Antioxidant and docking

INTRODUCTION

1,3- Oxazine constitute an important class of heterocyclic compounds because of their wide spectrum of biological properties, such as antimicrobial andantitubercular[1], anti-HIV[2], antimalarial[3], anticoagulant[4], anticonvulsant[5], antitubular[6], antithrombotic[7] and antitumour activities [8].

Inflammation is the reaction process of living tissues to stimuli induced by inflammatory agents, such as physical injuries, heat, microbial infections, and noxious chemical irritants, where the response of cells toward inflammation will lead to appear certain pathological indicators, such as redness, heat, swelling, and pain, sometimes with impaired physiological functions [9].

Important inflammatory mediators, such as prostaglandins, prostacyclin, and thromboxane, are produced through the prostanoid biosynthetic pathway from arachidonic acid using cyclooxygenase (COX) as a key enzyme [10]. The COX enzyme exists in two different forms, cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2). COX-2 is associated with inflammation and the resulting pain [11]. Thus, to inhibit the activity of the COX-2 enzyme, many non-steroidal anti-inflammatory drugs (NSAIDs) have been developed [12]. However, the use of such drugs results in many side effects such as, ulcerogenic, cardiovascular effects, etc [13]. Selective COX-2 drugs like celecoxib, etoricoxibetc is usually specific to inflamed tissue, and there is much less gastric irritation associated with COX-2 inhibitors, with a decreased risk of peptic ulceration .Therefore selective COX-2 inhibitors are preferred than traditional NSAIDS. However long term use of the above can have an effect on kidney, heart leading to thrombosis and stroke [14]. So there is always a need to develop newer anti-inflammatory agents with minimum side effects.

Schiff bases are biologically active compounds. They possess a lot of biological activities such as anticancer [15], antidepressant [16], antibacterial [17], anti-inflammatory [18], anti-tuberculosis [19], antimicrobial [20] etc.

Taking all the above facts into consideration it was thought to synthesize some oxazine and Schiff's bases of oxazine and also to compare the anti-inflammatory potency of the synthesized compounds.

EXPERIMENTAL SECTION

General information

All the melting points were determined in a Thermonik melting point apparatus and are uncorrected. The IR spectra of the synthesized compounds was recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400 -4000 using KBr pellets and the value of λmax were reported in cm-1. 1HNMR spectra was recorded on Amx - 400 MHz NMR spectrometer using DMSO and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as internal reference. 13C-NMR spectra was recorded on Amx - 400 MHz NMR spectrometer using DMSO and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as an internal reference. A mass spectrum was recorded on Mass spectrophotometer (model Shimadzu) by LC-MS 2010A. The purity of the compounds was checked by thin-layer chromatography on silica gel G plates of 0.5mm thickness as stationary phase and combination of n-hexane: ethyl acetate in different ratios as mobile phase. Elemental analysis were analysed by Thermo Finnigan Flash EA 1112 Series.

General method for synthesis of N-(4-acetyl-phenyl)-isonicotinamide (1)

In a round bottom flask isonicotinyl chloride dissolved in methanol was stirred well on ice bath. A solution of *p*-aminoacetophenone in methanol was added to above reaction mixture dropwise with constant stirring. After complete addition, stirring was continued for 15 minutes and then refluxed for one hour. The reaction mixture was cooled and the precipitate obtained was filtered, dried and purified from isopropyl alcohol
M.W:387, yield: 95%, M.P: 106-108^{0}C, Rf value: 0.79, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}): 3046 (NH str), 2954 (C-H Arstr) , 1725 (C=O str), C=N 1682 , 1408(C=C Arstr).

General method for synthesis of N-{4-[3-(substituted phenyl)-acryloyl]-phenyl}-isonicotinamide (2 a-e)

Equimolar quantity of N-(4-acetylphenyl)-isonicotinamideMKA and substituted aromatic benzaldehyde were dissolved in absolute alcohol and 40 % KOH solution was added slowly with stirring. After complete addition stirring was continued for 9 hours and kept overnight. The reaction mixture was poured in ice-water and acidified with 10 % HCl to obtain product. Crude product was recrystallized from ethanol. Reaction was monitored by TLC using different ratios of n-hexane and ethyl acetate

N-[4-3-(p-tolyl)-acryloyl-phenyl]-isonicotinamide 2a

M.W: 373, yield: 80%, M.P: 120-122^{0}C, Rf value: 0.81, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}):3160 (NH str), 2860 (Ar C-H str), 1700 (C=O str), 1500 (CH= CH str), 840 (Ar-CH_3str).

N-{4-[3-(3-chloro-phenyl)-acryloyl]-phenyl}-isonicotinamide 2b

M.W : 362 , yield: 80%, M.P: 130-132^{0}C , Rf value: 0.84,solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}):3382 (NH str), 2968 (Ar C-H str), 1712 (C=O str), 1642 (CH= CH str), 752 (Ar-Clstr).

N-{4-[3-(3-nitro-phenyl)-acryloyl]-phenyl}-isonicotinamide 2c

M.W: 373, yield: 75%, M.P: 126-128^{0}C, Rf value: 0.81, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}):3419 (NH str), 2918 (Ar C-H str), 1747 (C=O str), 1585 (CH= CH str), 1126 (Ar-NO_2 str).

N-{4-[3-(4-hydroxy-phenyl)-acryloyl]-phenyl}-isonicotinamide 2d

M.W: 344, yield: 77%, M.P: 136-138^{0}C, Rf value: 0.82, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}):3400 (b, OH str), 3053 (NH str), 2848 (Ar C-H str), 1714 (C=O str), 1645 (C=N str), 1622 (CH=CH str).

N-{4-[3-(3-methoxy-phenyl)-acryloyl]-phenyl}-isonicotinamide 2e

M.W: 358, yield: 82%, M.P: 122-124^{0}C, Rf value: 0.81, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}):3390 (NH str), 3056 (Ar C-H str), 2918 (Al CH str), 1705 (C=O str), 1620 (C=N str), 1527 (CH= CH str), 1012 (C-O-C str).

General method for the synthesis of N-{4-[2-amino-4-(substituted phenyl)-6H-[1,3] oxazine-6-yl]-phenyl}-isonicotinamide (3a-e)

Procedure

Equimolar quantity of **(2a-e)** and urea were dissolved in ethanolicNaOHand was stirred for 2-3 hours at room temperature followed by refluxing for 6 hours. The reaction mixture was poured into cold water with continuous stirring for one hourand was cooled at 0^o C for 12 hours. The precipitate obtained was filtered, washed and re-crystalized from ethanol.

N-[4-(2-amino-4-p-tolyl-6*H*-[1,3]oxazin-6-yl)-phenyl]-isonicotinamide 3a

M.W: 384, yield 78%, M.P: 86-88^{o}C, Rf value 0.80, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}): 3462 & 3341 (NH_2str), 3046 (C-H Arstr), 1611(C=O str), 1438 (C=N Arstr), 1120 (C-O-C str).
1H-NMR400 MHz, $CDCl_3$, δ-ppm): 7.92-6.45 (m,12H, ArH+1H of NH+1H_a of oxazine +solvent peak), 5.133 (s, 1H_b, oxazine nucleus), 2.48 (s, 2H, NH_2), 2.44 (s, 3H, CH_3)
13CNMR ($CDCl_3$, ppm): Ca-64.36, Cb-113.85, Cc-152.12, Cd-175.38, Ce-131.14, Ck and Cf- 131.38, Cj and Cg- 121.34, Ch- 130.85, Ci- 26.04, Cl-137.05, Cm and Cq-130.69, Cn- 127.18, Cp-127.41, Co-139.92, Cr-177.05, Cs- 143.35, Ct- 128.04, Cu-130.54, Cv-151.54, Cw-151.61
m/z:(M+1) 385
CHN: Found C= 71.26%, H= 5.49%, N=14.37%. Calculated C=71.86%, H=5.24%, N= 14.57%.

***N*-{4-[2-amino-4-(3-chloro-phenyl)-6*H*-[1, 3]oxazine-6-yl]-phenyl}-isonicotinamide 3b**

M.W: 404,yield 75%, M.P: 180-182^{o}C, Rf value 0.82, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm−1): 3425 & 3333 (NH_2 str),3058 (C-H Arstr), 1652 (C=O str), 1579 (C=N str), 1442 (C=C Arstr), 1178 (C-O-C str).
1H-NMR400 MHz, $CDCl_3$, δ-ppm): 9.25-9.2 (d, 2H, pyridine ring t,v), 7.93-7.91 (m, 4H, ArH j, i, h, f), 7.71-7.67 (d,2H, pyridine ring s,u), 7.29 (s, 1H, NH), 6.84 (s, 1H, oxazine a), 6.7-6.68 (m,4H, ArHm,l,o,p), 4.4 (s, 1H, oxazine b), 3.9 (s, 2H, NH_2))13CNMR ($CDCl_3$, ppm): Ca-60.98, Cb-105.42, Cc-143.25, Cd-163.02, Ce-139.91, Cf and Cg- 121.35,Cj- 137.42, Ch and Ci- 110.91, Ck- 131.11, Cl and Cp-128.12, Cm and Co-113.87, Cn- 130.87, Cq-165.25, Cr-151.57, Cs and Cu-120.39, Ct and Cv- 153.39.
m/z:(M+1) 405

CHN: Found C= 65.55%, H= 4.44%, N= 13.72%. Calculated C= 65.27%, H= 4.23%, N= 13.84%.

***N*-{4-[2-amino-4-(3-nitro-phenyl)-6*H*-[1,3]oxazine-6-yl]-phenyl}isonicotinamidemethane 3c**

M.W: 415, yield 72 %, M.P: 158-160^{o}C, Rf value 0.79, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm−1): 3474 & 3359 (NH_2 str), 3076 (C-H Arstr), 1592 (C=O str), 1500 (C=N str), 1438 (C=C Arstr), 1178 (C-O-C str).
1H-NMR400 MHz, $CDCl_3$, δ-ppm): 9.25 (s,2H, pyridine nucleus),8.42 (s, 1H, NH), 7.29-7.93 (m, 10H, ArH),6.84 (s, $1H_b$, oxazine nucleus), 4.40 (s, $1H_a$oxazine nucleus), 3.90 (s, 2H, NH_2).
13CNMR ($CDCl_3$, ppm): Ca-60.98, Cb-105.42, Cm and Co-110.91, Cf-113.87, Cs,Cu and Ch-120.39, Cl and Cp- 121.35, Cj-127.42, Ce- 128.12, Ci- 130.87, Cn-131.11, Ck-137.42, Cc- 139.91, Cr-143.25, Cg-151.57, Ct and Cv-153.39, Cd- 163.02, Cq- 165.25.
m/z::(M+1) 416

CHN: Found C=63.55%, H= 4.44%, N= 16.72%. Calculated C= 63.61%, H= 4.12%, N= 16.86%.

***N*-{4-[2-amino-4-(4-hydroxy-phenyl)-6*H*-[1,3]oxazine-6-yl]-phenyl}isonicotinamide 3d**

M.W: 386, yield 70 %, M.P: 160-162^{o}C, Rf value 0.74, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm−1): 3333 (b OH str), 3175 & 3065 (NH_2 str), 2765 (C-H Arstr), 1725(C=O str), 1586 (C=N str), 1408 (C= C Arstr), 1267 (C-O-C str).
^{1}H-NMR400 MHz, $CDCl_3$, δ-ppm): 7.914-6.443 (m, 12H, ArH+1H of NH+$1H_a$ of oxazine nucleus), 5.182 (s, $1H_b$, oxazine nucleus), 4.284 (s, 1H, OH), 2.442 (s, 2H, NH_2).
^{13}CNMR ($CDCl_3$, ppm): Ca-61.88, Cb-106.41, Cm and Co-111.90, Cf and Cj-113.12, Cs and Cu -120.33, Cl and Cp- 122.35, Cg-127.29, Ce-128.22, Ci- 131.87, Cn-132.11, Ck-137.22, Cc- 139.92, Cr-144.25, Ch-152.57, Ct and Cv-153.32, Cd- 163.22, Cq- 165.55.
m/z::(M-1) 385
CHN: Found C=68.23%, H= 4.39%, N= 14.57%. Calculated C= 68.38%, H= 4.70%, N= 14.50%.

***N*-{4-[2-amino-4-(3-methoxy-phenyl)-6*H*-[1,3]oxazine-6-yl]-phenyl}isonicotinamide 3e**

M.W: 400, yield 65 %, M.P: 168-170^{o}C, Rf value 0.78, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm−1): 3444 and 3341 (NH_2 str), 3058 (C-H Arstr), 1634, (C=O str), 1584 (C=N str), 1417 (C=C Arstr), 1058 (C-O-C str).
^{1}H-NMR400 MHz, $CDCl_3$, δ-ppm): 7.983-6.353 (m, 12H, ArH+1H of NH+$1H_a$ of oxazine nucleus), 5.140 (s, $1H_b$, oxazine nucleus), 3.163 (s, 3H, OCH_3), 2.242 (s, 2H, NH_2).
^{13}CNMR ($CDCl_3$, ppm): Ci-54.04, Ca-63.54, Cb-112.95, Ct and Cu-120.99,Cn and Cp-127.15,Cj-127.45 , Ch-130.54, Cm and Cq-130.69,Cs -130.85, Ck and Cf- 131.14, Ce-131.38, Co- 144.35, Cc- 151.44, Cl-151.61, Cv and Cw-153.12, Cg- 160.30, Cd-168.38, Cr-172.05.
M/z:(M+1) 401
CHN: Found C=68.86%, H= 5.09%, N= 13.97%. Calculated C= 68.99%, H= 5.03%, N= 13.99%.

General method for the synthesis of N-(4-{4-(substituted phenyl)-2-[(4-fluro-benzylidene)-amino]-6H-[1,3] oxazine-6-yl}-phenyl)-isonicotiamide (4a-e)

Procedure:

Mixture of**(3a-e)(0.1M)** and ***p*-fluorobenzaldehyde (0.2M)** were dissolved in absolute ethanol and were stirred for 12 hours using conc H_2SO_4 as a catalyst (2drops). The reaction mixture was poured in crushed ice. The solid was filtered and recrystallized from ethanol.

N-(4-{2-[(4-fluoro-benzylidene)-amino]-4-*p*-tolyl-6H-[1,3]oxazine-6-yl}-phenyl)-isonicotinamide 4a

M.W: 490, yield 75 %, M.P: 132-134 ^{oC}, Rf value: 0.86, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}): 2916, 2852 (ArCHstr), 1643 (C=O str), 1600 (C=N str), 1219 (C-O-C str), 815 (Ar-CH_3)
^{1}H-NMR400 MHz, DMSO-*d6*, δ-ppm): 8.45 (s,2H,NH+CH of imine carbon), 8.12-8.10 (d, 2H, pyridine ring z_3&z_4), 7.94-7.83 (d, 2H, pyridine ring z_1&z_2), 7.60-7.54 (m, 4H, ArHu,t,w,x), 7.30-7.19 (m, 8H, ArHm,n,o,q,g,k,i,h), 6.73-6.71 (d, 1H, oxazine b), 4.17 (s,1H, oxazine a), 2.19 (s, 3H, CH_3).
^{13}CNMR (DMSO, ppm): Cr-30.93, Ca-69.70, Ck& Ci-113.94, Cb-115.99, Cw& Cu-116.21, Cz_1& Cz_2-120.92, Cn& Cm-121.05, Cx& Ct-128.29, Cf-128.49, Co & Cq-129.61, Cg & Ch-129.72, Cl-129.94, Cs-131.03, Cv-131.10, Cz-131.19, Cc132.24, Cp-135.78, Cd-141.07, Ce-143.22, Cj-144.71, Cz_3& Cz_4-155.84, Cy-160.07.
m/z :(M+1) 491, (M-1) 489
CHN: Found C= 73.32%, H= 4.74%, N= 11.51%. Calculated C= 73.46%, H= 4.73%, N= 11.42%.

N-(4-{4-(3-chlorophenyl)-2-[(4-fluoro-benzylidene)-amino]-6H-[1,3]oxazine-6-yl}-phenyl)-isonicotinamide 4b

M.W: 510, yield 78 %, M.P: 128-130^{o}C, Rf value: 0.84, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm^{-1}): 2920 (ArCHstr), 1597 (C=O str), 1303 (C=N str), 1228 (C-O-C str), 831 (Ar-Cl)
^{1}H-NMR400 MHz, DMSO-*d6*, δ-ppm): 8.45 (s, 2H, NH+CH imine carbon), 8.12-8.10 (d, 2H, pyridine ring z_2 and z_1), 7.96-7.94 (d, 2H, pyridine ring y,z), 7.76-7.21 (m,12H, ArH), 6.73-6.71 (d, 1H, oxazine b), 4.21 (s, 1H, oxazine a).
^{13}C-NMR (DMSO, ppm): Ca-69.59, Ck& Ci-113.97, Cb-116.02, Cv& Cu-116.23, Cy & Cz-121.02, Cn-123.10, Cm-123.30, Cs & Ct-126.68, Cp-126.84, Co-127.68, Cg & Ch-127.87, Cf-128.30, Cq-129.88, Cl-130.04, Cr-130.12, Cz_3-130.23, Cx-130.32, Cc-131.17, Cz_1& Cz_2-134.88, Cd-137.23, Ce-141.38, Cj-142.82, Cw-151.29.
m/z: (M-1) 509
CHN: Found C= 68.32%, H= 3.86 %, N= 10.89 %. Calculated C= 68.17%, H= 3.95%, N= 10.97 %.

N-{4-[2-[(4-fluorobenzylidene)-amino]-4-(3-nitro-phenyl)-6H-[1,3]-oxazin-6-yl]-phenyl}-isonicotinamide 4c

M.W:521, yield: 80 %, M.P: 126^{o}C, Rf value 0.87, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm−1): 2924 (ArCHstr), 1625 (C=O str), 1525 (C=N str), 1226 (C-O-C str), 827 (Ar-NO_2)
^{1}H-NMR400 MHz, DMSO-*d6*, δ-ppm): 9.99 (s, 2H, NH+CH imine), 8.52 (s, 2H, pyridine ring z_1& z), 8.25-8.24 (d, 2H, pyridine ring z_2& z_3), 7.98-7.61 (m, 12H, ArH), 6.74-6.73 (d, 1H, oxazine b), 4.24 (s, 1H, oxazine a).
^{13}CNMR (DMSO, ppm): Ca-66.25, Cb-114, Cv& Cu-117.08, Cm-120.87, Cy & Cz-122.04, Cf-124.19, Cs & Ct-124.82, Cr & Co-129.91, Cg & Ck-131.26, Cn-134.26, Cp-139.97, Ci& Ck-140.68, Cc & Cx-142.92, Cq-144.27, Cz_2& Cz_1-150.87, Ce-160, Cj-165.67, Cw-168.68.
m/z : (M-1) 520
CHN: Found C= 66.78%, H= 3.81 %, N= 13.35 %. Calculated C= 66.79%, H= 3.87%, N= 13.43 %.

N-{4-[2-[(4-fluorobenzylidene)-amino]-4-(4-hydroxy-phenyl)-6H-[1,3]-oxazin-6-yl]-phenyl}-isonicotinamide 4d

M.W: 492, yield 75 %, M.P: 122-124^{o}C, Rf value 0.84, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm−1): -3442 (b, OH +NH str),3223 (NH str), 2954 (Ar C-H str), 1664(C=O str),1585 (C=N str), 1222 (C-O-C str), 785 (Ar-F str).
^{1}H-NMR400 MHz, DMSO-*d6*, δ-ppm): 8.45 (s,2H,NH+CH of imine carbon), 8.12-8.10 (d, 2H, pyridine ring z_3&z_4), 7.96-7.83 (d, 2H, pyridine ring z_1&z_2), 7.60-7.54 (m, 4H, ArHu,t,w,x), 7.30-7.19 (m, 8H, ArHm,n,o,q,g,k,i,h), 6.73-6.71 (d, 1H, oxazine b),5.15(s, 1H, OH), 4.19 (s,1H, oxazine a).
^{13}CNMR (DMSO, ppm): Ca-67.25, Cb-113, Ct & Cv-116.08, Cn& Cm-120.87, Cz& Cz1-122.04, Cf-124.19, Cs & Cw-124.82, Ch, Cj, Co and Cp-129.91, Cg and Ck-131.26, Cl-135.26, Cr-139.97, Cc-140.68, Cy142.92, Cq-145.27, Cz2 and Cz3-150.87, Cd-156.27, Ce-160, Ci-165.67, Cx-167.12
m/z:(M-1) 491
CHN: Found C=70.78%, H= 4.81%, N= 11.35%. Calculated C= 70.72%, H= 4.30%, N= 11.38%.

N-{4-[2-[(4-fluorobenzylidene)-amino]-4-(3-methoxy-phenyl)-6H-[1,3]-oxazin-6-yl]-phenyl}-isonicotinamide 4e

M.W: 506, yield 77 %, M.P: 130-132^{o}C, Rf value 0.82, solvent ratio: ethyl acetate: n-hexane (7:3)
IR (KBrυmax cm−1): 2223 (NH str), 2978 (Ar C-H str), 2918 (Al CH str), 1712 (C=O str), 1633 (C=N str), 1249 (C-O-C str), 744 (Ar-F str).
^{1}H-NMR400 MHz, DMSO-*d6*, δ-ppm): 8.55 (s,2H,NH+CH of imine carbon), 8.13-8.12 (d, 2H, pyridine ring z_3&z_4), 7.96-7.50 (d, 2H, pyridine ring z_1&z_2), 7.40-7.30 (m, 4H, ArHu,t,w,x), 7.28-7.21 (m, 8H, ArHm,n,o,q,g,k,i,h), 6.73-6.72 (d, 1H, oxazine b), 4.28 (s,1H, oxazine a), 3.31 (s, 3H, OCH_3).
^{13}CNMR (DMSO, ppm): Cr-54.93, Ca-70.70, Ck and Ci-112.94, Cb-115.92, Cw& Cu-116.22, Cz1 & Cz2-120.92, Cn& Cm-122.02, Cx and Ct-128.29, Cf-128.49, Co and Cp-129.61, Cg and Ch-129.72, Cl-129.94, Cs-130.03, Cv-131.10, Cz-131.19, Cc-133.24, Cq-136.78, Cd-141.07, Ce-143.22, Cj-144.71, Cz3 and Cz4-156.84, Cy-161.07
m/z:(M+1) 507
CHN: Found C=71.32%, H= 4.86%, N= 11.89%. Calculated C= 70.72%, H= 4.30%, N= 11.38%.

RESULTS AND DISCUSSIONS

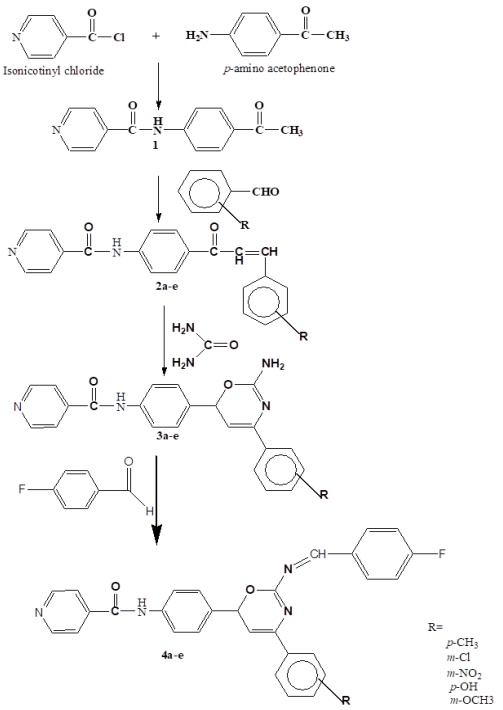
The synthetic methodology involved the synthesis of oxazine and Schiff's bases of oxazine [21]. In **step1** the reaction between isonicotinylchloride and 4-amino acetophenonein methanol as a solvent, yielded N-(4-acetyl-phenyl) isonicotinamide**.** The presence of CONH was established by IR absorption peak at 3046 cm^{-1} and ketone group at 1725 cm^{-1} [22].

Step2 derivatives **(2a-e)** were obtained by reaction of 1 with substituted aromatic aldehydes by the ClaisenSchmidth reaction in the presence of ethanolic 40%KOH to form the respective chalcones. The weak absorption peaks at 1500cm^{-1} suggest the presence of olefins carbon, strong stretching peaks at 2860 cm^{-1} indicate =C-H (vinyl hydrogen), the keto of propenone was found at 1700 cm^{-1}.

Step 3 involved the cyclization of chalcone moiety to oxazine ring on treatment with urea which was dissolved in ethanolicNaOH. The IR absorption peak at 3462 cm^{-1} suggest primary amino group .Strong absorption peaks at 1438- cm^{-1} supported the presence of –C=N of oxazine.The formation of oxazine moiety is also confirmed by a broad singlet peak at δ 4.4 sp^2 C-H and a sharp singlet at δ 6.84 for sp^3. The cyclic C-O-C carbon peaks are observed at δ60 & δ105 supporting the formation of oxazine ring. The other sp^2 carbons of oxazine were seen δ 143 and δ 163 respectively and the rest of the aromatic protons fall within the range of 113-153. The presence of secondary amide isalso established from ^{1}H NMR at δ 7.9 for NH proton and^{13}C NMR oxo carbon at δ 165; multiplet peaks seen at δ 6.8 and 7.9 indicate aryl hydrogens. The presence of a primary amino group is observed at δ 3.9. Mass spectra of compounds are in agreement with their molecular formula. The structures are also confirmed from CHN analysis wherein the calculated and experimental values of the elements present in the compounds do not differ by ±0.5.

In **step 4** the oxazine derivatives were subjected to Schiff's base reaction using p-flurobenzaldehyde in ethanolic medium using conc H_2SO_4 as catalyst. The IR spectra clearly show the disaapearence of NH_2 peak at 3600-3200 cm^{-1}. The imine H is seen as a singlet in the range of δ 9.92-8.45 along with NH peak which was absent in the oxazine molecule indicating that the oxazine moiety undergo Schiff's reaction with p-

flurobenzaldehyde in ethanolic medium. The ^{13}C-NMR also points the presence of imine carbon in the range of δ 143-141. Mass spectra of compounds are in agreement with their molecular formula. The structures are also confirmed from CHN analysis wherein the calculated and experimental values of the elements present in the compounds do not differ by ± 0.5.



Scheme 1: Synthesis of N-{4-[2-amino-4-(substituted phenyl)-6H-[1, 3] oxazine-6-yl]-phenyl}-isonicotinamide (3a-e) and N-(4-{4-(substituted phenyl)-2-[(4-fluro-benzylidene)-amino]-6H-[1,3] oxazine-6-yl}-phenyl)-isonicotiamide (4a-e)

ANTI-INFLAMMATORY ACTIVITY

In vitro anti-inflammatory activity

Bovine serum albumin assay [23] (Table 1).

$$\% \text{ inhibition } = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Table 1: In-Vitro anti-inflammatory activity.

Sl No.	Comp Code	% Inhibition	
		100 µg/ml	200µg/ml
1	3a	89	90
2	3b	70	72
3	3c	71	74
4	3d	76	77
5	3e	78	79
6	4a	84	86
7	4b	79	80
8	4c	77	79
9	4d	82	83
10	4e	84	85
Standard	Indomethacin	90	91

In vitro antioxidant activity

(a). Screening of antioxidant activity by DPPH method [24](Tables 2 & 3)

(b). Nitric oxide free radical scavenging activity [25]

% inhibition is calculated by following formula

$$\% \text{ inhibition } = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Table 2: DPPH free radical scavenging activity.

Sl No.	Comp Code	% Inhibition		
		10 µg/ml	50 µg/ml	100 µg/ml
1	3a	70	72	74
2	3b	63	64	68
3	3c	70	72	73
4	3d	87	88	88
5	3e	88	89	93
6	4a	80	82	84
7	4b	72	73	74
8	4c	73	74	75
9	4d	94	94	94
10	4e	80	82	87
11	Ascorbic acid	97	98	99

Table 3: Nitric oxide free radical scavenging activity.

Sl No.	Comp Code	% Inhibition		
		10 µg/ml	50 µg/ml	100 µg/ml
1	3a	45	48	48
2	3b	41	42	42
3	3c	44	45	46
4	3d	52	52	58
5	3e	53	58	60
6	4a	47	47	49
7	4b	43	43	45
8	4c	48	49	49
9	4d	60	62	63
10	4e	61	61	64
11	Ascorbic acid	40	55	62

Anti-inflammatory Activity

Anti-inflammatory drugs are judged by their effect on the pain, stiffness or swelling of the affected part, the action on swelling being the most objectively observable and therefore the most important. Thus a drug that reduces inflammatory swelling could act as diminishing their ceased vascular permeability.

The anti-inflammatory studies of the synthesized compounds were carried out using BSA methods using indomethacine as the positive control.BSA on heating undergoes denaturation and express antigens associated with type III hypersensitive reaction and which are related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus. Thus the assay can be applied for the discovery of these drugs which can stabilize the protein from denaturation process. Good activity was observed with BSA method which also is dose dependant. Compounds 3a, 3d and 3e in the oxazine series (having *p*-CH_3, *p*-OH and *m*-OCH_3 respectively) have shown very significant effect which may be attributed due to the electron donating substituent. Further it was seen that activity increased when the oxazine molecules underwent Schiff's base reaction.

Antioxidant Activity

Low level of anti-oxidant or inhibition of the anti-oxidant enzymes cause oxidative stress and may damage or kill cells; as oxidative stress might be an important part of many human diseases. Antioxidants terminate these chain reactions by being oxidized.

The anti-oxidant studies of the synthesized compounds were carried out using NO and DPPH method. NO is a strong inhibitor of physiological processes of smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems.NO rapidly degrades to nitrate and nitrite. In aqueous solution sodium nitroprusside generates NO at physiological pH. Griess reagent is used to measure nitrite but not nitrate which can be predicted spectrophotometrically. The test sample or compounds at tested doses reduces the nitrates to nitrite prior to the addition of Griess reagent. The percentage reduction of nitric oxide or accumulation of nitrite, thus indicating the scavenging activity of the test sample.

DPPH is the simplest and most widely reported method for screening anti-oxidant activity in foods and many plant drugs. DPPH takes one electron in the presence of a free radical scavenger, leads to discoloration which is stoichiometrically related to the number of electrons gained, which is observed by decrease in absorption. In this assay, the purple chromogen radical 2,2-diphenyl -1-picrylhydtazyl (DPPH) is reduced by reducing compounds to the corresponding pale yellow hydrazine.

The observation revealed that all compounds have shown significant effect; compounds having electron releasing group such as methyl, hydroxyl

and methoxy groups on aryl ring have shown very significant effect by both the methods. Additional increase in activity is further observed with Schiff's base derivatives.

Very good antioxidant effect of synthesized compounds suggest their free radical scavenging action may be responsible for their anti-inflammatory effect, where inflammation is due to stress related leading to the release of free radical.
Overall the anti-oxidant and anti-inflammatory activity may be attributed because of oxazine moiety, primary amino group, imide linkage with flurosubstitutent and secondary amide linkage.

Docking Study

Molecular docking is a significant computational method used to forecast the binding of the ligand to the receptor binding site by varying position and conformation of the ligand keeping the receptor rigid. Docking also helps in understanding the various interactions between ligand and enzyme in the active site in detail.

AutoDockVina is a new open source program for drug discovery, molecular docking and virtual screening, offering multi-core capability, high performance and enhanced accuracy and ease of use. AutoDockVina significantly improves the average accuracy of the binding mode predictions.

Anti-inflammatory

The synthesized compounds were docked into the active site of COX-2 containing celecoxib (PDB: 3LN1) [26](Tables 4-7).

Table 4: N-{4-[2-amino-4-(substituted phenyl)-6H-[1,3] oxazine-6-yl]-phenyl}-isonicotinamide (3a-e) and N-(4-{4-(substituted phenyl)-2-[(4-fluro-benzylidene)-amino]-6H-[1,3] oxazine-6-yl}-phenyl)-isonicotiamide (4a-e) with their binding energy scores (Kcal/mol)and H-bonds interactions against COX-2 (PDB: 3LN1).

Ligand Code	Binding energy score (Kcal/mol)	H-bond Interacting Residues
3a	-9.8	Trp C373, Asn C368
3b	-8.9	Trp C373, Asn C368
3c	-8.4	His C 193
3d	-9.1	Trp C373, Asn C368
3e	-9.1	Trp C373, Asn C368
4a	-10.8	His C372, C374, Gln C189
4b	-9.8	Trp C373, Thr C192
4c	-10.2	Gln C275, His C372,C374 Tyr C371
		Vanderwaal's interactions: Gln C189
4d	-10.3	His C372, C374, Gln C189
4e	-10.4	His C372, C374, Gln C189
Reference Ligand	-13.1	PheC504,ArgC499, HisC75,LeuC338, SerC339, GlnC178

Table 5: N-{4-[2-amino-4-(substituted phenyl)-6H-[1,3] oxazine-6-yl]-phenyl}-isonicotinamide (3a-e) and N-(4-{4-(substituted phenyl)-2-[(4-fluro-benzylidene)-amino]-6H-[1,3] oxazine-6-yl}-phenyl)-isonicotiamide (4a-e)with their binding energy scores (Kcal/mol)and H-bonds interactions against selective COX-2(PDB: 1CX2)[27].

Ligand Code	Binding energy score (Kcal/mol)	H-bond Interacting Residues
3a	-9.9	Leu C472
3b	-9.3	-
3c	-9.3	Ala C199
3d	-9.6	Arg C469, Tyr C122
3e	-9.5	Tyr C122, Arg C469
4a	-10	-
4b	-9.3	Asn C43, Arg C61

4c	-9.3	Arg C44, Tyr C122
4d	-9.7	-
4e	-9.8	Glu C465, Lys C83
Reference Ligand	-8	Ala C202, Trp C387

Table 6: Calculation of molecular properties of N-{4-[2-amino-4-(substituted phenyl)-6H-[1,3] oxazine-6-yl]-phenyl}-isonicotinamide (3a-e) and N-(4-{4-(substituted phenyl)-2-[(4-fluro-benzylidene)-amino]-6H-[1,3] oxazine-6-yl}-phenyl)-isonicotiamide (4a-3).

Comp Code	miLog P	TPSA	Natoms	MW	nON	nOHNH	nviolations	nrotb	Volume
3a	2.96	89.61	29	384	6	3	0	4	347.59
3b	3.16	898.61	29	404	6	3	0	4	344.56
3c	2.44	135.44	31	415	9	3	0	5	354.36
3d	2.03	109.84	29	386	7	4	0	4	339.04
3e	2.54	98.84	30	400	7	3	0	5	356.57
4a	5.17	75.95	37	490	6	1	1	6	435.9
4b	5.37	75.95	37	510	6	1	2	6	432.87
4c	4.65	121.78	39	521	9	1	1	7	442.67
4d	4.24	96.18	37	492	7	2	0	6	427.36
4e	4.75	85.19	38	506	7	1	1	7	444.88

Online Toxicity Prediction

Using OSIRIS softwarehttps://www.organic-chemistry.org/prog/peo/ assessed on 30-07-2020 (Table 7).

Table 7: Online Toxicity Prediction.

Comp Code	Mutagenic	Tumorigenic	Irritant	Reproductive Effective
3a	-	-	-	-
3b	-	-	-	-
3c	-	-	-	-
3d	-	-	-	-
3e	-	-	-	-
4a	-	-	-	-
4b	-	-	-	-
4c	-	-	-	-
4d	-	-	-	-
4e	-	-	-	-

Docking Studies

Anti-inflammatory

Molecular docking studies of synthesized oxazine and Schiff's bases of oxazine on protein cyclooxygensase target especially selective COX-2 (PDB ID 3LN1), exhibited binding affinities in the range of -8.9 to -10.8 kcal/mol whereas co crystallised ligand which has Celecoxib as the standard exhibited the binding affinity of -13.1 kcal/mol (Table 4).Almost all the derivatives showed good binding affinity with various amino acids prominently being ASN, GLN, TRP, HIS, THR, TYR. It was evident that mostly the H of the amino group, O and H of amide, O of oxazine ring, H of pyridine ring formed hydrogen bonds with the amino acids. This is also proved by the *invitro* anti-inflammatory activity. The fact that transformation of oxazine into Schiff's bases has increased the anti-inflammatory activity is further proved by docking studies too as **4a-e** have quite high binding energy scores than the**3a-e**. The assumption which has been proposed for the enhancement of anti-inflammatory activity for **3a**, **3d** and **3e** could not be proved by docking studies as NO_2 group is electron withdrawing group but has shown the highest docking score of -10.3 kcal/mol which may give an idea that probably the activity is due to the presence of pyridine, oxazine and imine group (Schiff's bases) and is not dependent on the substitutents present in the 4-phenyl ring.

These derivatives were also docked into the active site of selective COX-2 inhibitir (PDB ID 1CX2). The binding energies ranged from -9.3 to -10 kcal/mol which were quite high than the co-crystallized ligand which exhibited binding at -8 kcal/mol (Table 5). But some of the compounds failed to form hydrogen bonds with the amino acid residues of the protein which is a prerequisite for any biological activity. The amino acid which was involved in H-bonds was LEU, ALA, ARG, TYR, ASN, GLU, LYS whereas the standard formed H-bonds with ALA and TRP.

CONCLUSION

The titled Schiff's bases of oxazine were obtained by reaction of *p*-amino acetophenone with isonicotinyl chloride to obtain the secondary amides which undergone Claisen Schmidth condensation reaction with substituted aldehydes to form chalcones. These chalcones were cyclized with urea to form oxazine which undergone reaction with *p*-fluorobenzaldehyde to form the corresponding Schiff's bases. All the compounds were obtained from moderate to good yields. All the titled compounds showed good antioxidant and anti-inflammatory activity. The synthesized compounds were docked against protein receptors for screening of anti-inflammatory activities. Our synthesized compounds were also found to be non-toxic using online toxicity prediction using OSIRIS software. Oxazine derivatives may be favoured compared to their corresponding Schiff's base as most of them do not have drug like property as they failed to pass the Lipinski Rule of 5.

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