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Synthesis of new imidazolones and their biological evaluation as COX-2 inhibitors.

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ABSTRACT

New series of imidazolones were designed and synthesized and evaluated against both COX-1 and COX-2 enzymes. Based on the outcome of *in vitro* COX assay most of the derivatives showed selective COX-2 inhibitory activity specifically compounds **IIa and IVb** with IC_{50} values 0.12 and 0.19 µM compared to celecoxib with IC_{50} value of 0.05 µM and diclofenac sodium with IC_{50} value of 0.8 µM on COX-2 enzyme.

Keywords: Imidazolone; COX-1; COX-2; Synthesis; anti-inflammatory.

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INTRODUCTION

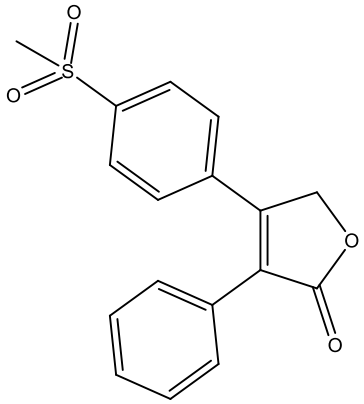
The use of non-steroidal anti-inflammatory drugs(NSAIDs) for the treatment of inflammation and pain is often accompanied by gastrointestinal ulcerations and bleeding.[1]It was recognized that selective inhibitors of the inducible form COX-2 expressed mainly in inflammatory cells could provide anti-inflammatory agents devoid of the undesirable effects associated with classical, nonselective NSAIDs. [2]

Celecoxib (Celebrex)™ was the first selective COX-2 inhibitor (coxibs) that appeared on the world markets in 1999 as a safer replacement for NSAIDs (non-selective COX-1/COX-2 inhibitors) as it causes less gastrointestinal complications.[3]Many other compounds widely used in the treatment of pain and inflammation, such as rofecoxib, valdecoxib and indomethacin. [4]**Fig 1**

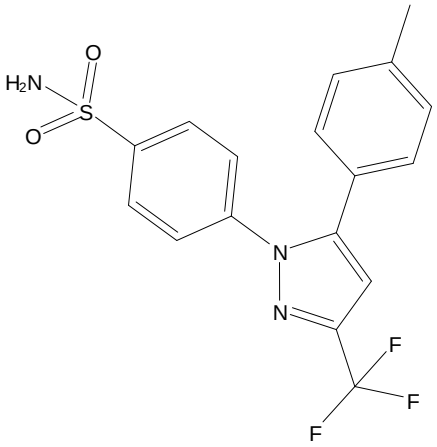
The literature surveys depicts that imidazole derivatives show various pharmacological activities such as anti-viral, anti-inflammatory and analgesic, anti-depressant, anti-fungal and anti-bacterial, anti-cancer, anti-tubercular and anti-leishmanial activity.[5] Some reported imidazolones bearing sulphonyl group were reported as potent COX-2 inhibitors (1 &2). [6]On the other hand, some imidazole derivatives with p- phenyl substitution showed high anti-inflammatory and anti-nociceptive activity (3&4). [7,8]A series of 2,4-diaryl-5(4H)- imidazolone derivatives (5) showed a potent anti-inflammatory activity. [9]

From the above findings it was useful to synthesis on well-established structural features of selective COX-2 inhibitors new imidazolone derivatives either substituted or non-substituted with sulphonyl group to investigate their COX-2 inhibitory activity.

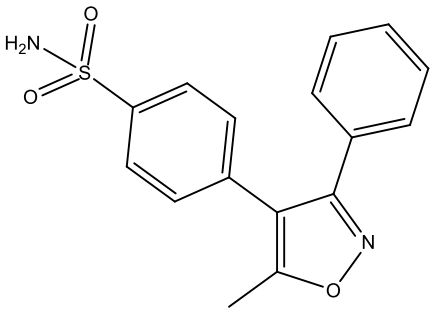
Fig 1: Structure of some NSAIDs and COX inhibitors



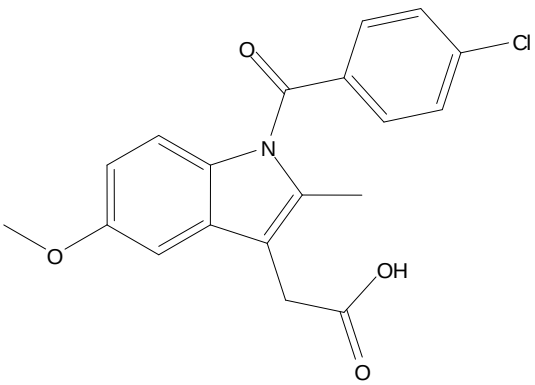
Rofecoxib



Celecoxib



Valdecoxib



Indomethacin

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MATERIALS AND METHODS

Chemistry

Melting points were determined on Stuart apparatus and the values given were uncorrected.

IR spectra were determined on Shimadzu IR 435 spectrophotometer at the Faculty of Pharmacy, Cairo University, Egypt. Using KBr discs (values were represented in cm^{-1}).^{1}HNMR spectra were recorded on Varian Gemini 300 MHz spectrophotometer at National Research Centre (NRC) Labs., Egypt and Bruker 400 MHz at Faculty of Pharmacy, Cairo University, Egypt. Using TMS as internal standard. Chemical shift values were recorded in ppm on δ scale.

^{13}CNMR spectra were recorded on Varian Gemini 300 MHz spectrophotometer at National Research Centre (NRC) Labs, Egypt. Using TMS as internal standard. Chemical shift values were recorded in ppm on δ scale. Mass spectra were recorded on EI-MS Hewlett Packard 5988 spectrometer at National Research Centre (NRC) Labs and Shimadzu QP-2010 Plus Micro Analytical Center, Cairo University, Cairo, Egypt. Elemental analyses were carried out at the Micro analytical Center, Alazhar University, Egypt. Progress of the reactions was monitored using TLC aluminum sheets precoated with UV fluorescent silica gel (Merck 60F 254) and were visualized using UV lamp.

Experimental

Oxazolone derivatives (**I_{a-g}**) was synthesized according to reported procedure [10]

General procedure for the synthesis of (II_{a-g}):

An equimolar Mixture of **I_{a-g}** (0.016 mol) and Sulphanilamide (0.016 mol) was heated under reflux in acetic acid (20 ml) in presence of fused anhydrous Sodium acetate (2.44 mmol,0.2gm) for 6 h. The solid formed after cooling was filtered, washed with acetic acid, dried and crystallized from acetic acid.

4-(4-Benzylidene-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl) benzenesulfonamide (II_a) :

Mp 225°C, yield: 70 % , IR (KBr) cm^{-1}: 3348, 3302 (NH_2), 3105 (CH aromatic), 2931(CH aliphatic), 1666 (C=O), 1373, 1095 (SO_2). ^{1}H NMR (DMSO) δ: 7.24 (m, 5H, Phenyl), 7.68(t, 1H, Pyridine C5), 7.89 (s, 1H, =CH), 8.60 (d, 4H, Phenyl), 8.90 (t, 2H, Pyridine C4, 6), 9.10(s, 1H, Pyridine C2), 10.54 (s, 2H, NH_2, D_2O exchangeable) ppm. ^{13}C NMR (DMSO) δ: 119.81 (Phenyl C2,3,5,6), 126.49 (CH=C),127.14 (CH=C), 138.49 (Phenyl C2`,3`,4`,5`,6`), 142.57 (Phenyl C 1`,1,6), 146.43(imidazole C=N), 148.64(Pyridine C3,4,5), 165.16 (Pyridine C2,6), 169.55 (C=O). Anal.Calcd for $C_{21}H_{16}N_4O_3S$: C, 62.36; H, 3.99; N, 13.85. Found: C, 62.49; H, 4.02; N, 13.99.

4-(4-(4-Chlorobenzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl) benzenesulfonamide (II_b) :

Mp 232°C, yield: 66 % , IR (KBr) cm^{-1}: 3369, 3304 (NH_2), 3100 (CH aromatic), 2931 (CH aliphatic), 1658 (C=O), 1330, 1097 (SO_2).^{1}H NMR (DMSO) δ: 7.23 (s, 1H, =CH), 7.74(m, 8H, Phenyl), 8.46 (t, 1H, Pyridine C5), 8.88 (m, 2H, Pyridine C4, 6), 9.15 (s, 1H, Pyridine C2), 10.34 (s, 2H, NH_2, D_2O exchangeable) ppm. Anal.Calcd for $C_{21}H_{15}ClN_4O_3S$: C, 57.47; H, 3.44; N, 12.77. Found: C, 57.71; H, 3.46; N, 12.89.

4-(4-(4-Hydroxybenzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)benzenesulfonamide (II_c) :

Mp 251°C, yield: 60 % , IR (KBr) cm^{-1}: 3369 (OH), 3290, 3207 (NH_2), 2991 (CH aliphatic), 1714 (C=O), 1330, 1097 (SO_2). ^{1}H NMR (DMSO) δ: 5.41 (s, 1H, OH, D_2O exchangeable), 7.21 (s, 1H, =CH), 7.70 (m, 4H, Phenyl), 7.81(m, 4H, Phenyl), 8.55 (t, 1H, Pyridine C5), 8.91 (t, 2H, Pyridine C4, 6), 9.14 (s, 1H, Pyridine C2), 10.4 (s, 2H, NH_2, D_2O exchangeable) ppm. MS: m/z (% abundance) 420 (M^+,0.18%), 57 (C_2H_3NO, 100%). Anal.Calcd for $C_{21}H_{16}N_4O_4S$: C, 59.99; H, 3.84; N, 13.33. Found: C, 60.17; H, 3.91; N, 13.57.

4-(4-(4-Methoxybenzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)benzenesulfonamide (II_d) :

Mp 235°C, yield: 70 % , IR (KBr) cm^{-1}:3369, 3352 (NH_2), 3182 (CH aromatic), 2991(CH aliphatic), 1669 (C=O), 1373, 1095 (SO_2). ^1H NMR (DMSO) δ: 2.46 (s, 3H, OCH_3), 7.21 (s, 1H, =CH),7.72 (m, 4H, Phenyl), 7.80 (m, 4H, Phenyl), 8.54 (t, 1H, Pyridine C5), 8.90 (d, *j=3.9*, 2H, Pyridine C4, 6), 9.13 (s, 1H, Pyridine C2), 10.47 (s, 2H, NH_2, D_2O exchangeable) ppm. Anal.Calcd for $C_{22}H_{18}N_4O_4S$: C, 60.82; H, 4.18; N, 12.9. Found: C, 60.98; H,4.24; N, 13.08.

4-(4-(4-(Dimethylamino)benzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)benzenesulfonamide (II_e) :

Mp 240°C, yield: 58 %, IR (KBr) cm^{-1}: 3369, 3290 (NH_2), 3107 (CH aromatic), 2960 (CH aliphatic), 1658 (C=O), 1371, 1097 (SO_2). ^1H NMR (DMSO) δ: 3.0 (s, 6H, $2CH_3$), 7.13 (s, 1H, =CH), 7.68(m, 4H, Phenyl), 7.72 (m, 4H, Phenyl), 8.65 (t, 1H, Pyridine C5), 8.96 (d, *j=2.88*, 2H, Pyridine C4, 6), 9.11 (s, 1H, Pyridine C2), 10.52 (s, 2H, NH_2, D_2O exchangeable) ppm. ^{13}C NMR (DMSO) δ: 40.68 ($2CH_3$), 126.79 (CH=C),129.48 (CH=C), 138.4 (Phenyl C2,3,5,6), 142.82 (Phenyl C2`,3`,4`,5`,6`), 143.38 ( Phenyl C 1`,1,4), 145.55(Pyridine C3,4,5), 148.01(Pyridine C2,6), 164.75 (imidazole C=N), 169.48 (C=O). Anal.Calcd for $C_{23}H_{21}N_5O_3S$: C, 61.73; H, 4.73; N, 15.65. Found: C, 61.94; H,4.81; N, 15.82.

4-(4-(2-hydroxybenzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)benzenesulfonamide (II_f) :

Mp 246°C, yield: 63 % , IR (KBr) cm^{-1}: 3309 (OH), 3292, 3207(NH_2), 3111 (CH aromatic), 2933 (CH aliphatic), 1658 (C=O), 1330, 1097 (SO_2). ^1H NMR (DMSO) δ: 5.5 (s, 1H, OH, D_2O exchangeable,), 7.23 (s, 1H, =CH), 7.71 (m, 4H, Phenyl), 7.87 (m, 4H, Phenyl), 8.63 (t, 1H, Pyridine C5), 8.93 (d, *j=3.45*, 2H, Pyridine C4, 6), 9.16 (s, 1H, Pyridine C2), 10.41 (s, 2H, NH_2 , D_2O exchangeable) ppm. Anal.Calcd for $C_{21}H_{16}N_4O_4S$: C, 59.99; H, 3.84; N, 13.33. Found: C, 60.16; H, 3.89; N, 13.54.

4-(5-oxo-2-(pyridin-3-yl)-4-(pyridin-3-ylmethylene)-4,5-dihydro-1*H*-imidazol-1-yl)benzenesulfonamide (II_g) :

Mp 233°C, yield: 40 %, IR (KBr) cm^{-1}: 3367, 3296 (NH_2), 3094 (CH aromatic), 2990 (CH aliphatic), 1697 (C=O), 1330, 1095 (SO_2). ^1H NMR (DMSO) δ: 7.23 (s, 1H, =CH), 7.69(m, 4H, Phenyl), 8.20 (t, 2H, Pyridine C5, 5\), 8.75 (d, *j=2.28*, 4H, Pyridine C4, 4\, 6, 6\), 9.03 (s, 2H, Pyridine C2, 2\), 10.47 (s, 2H, NH_2, D_2O exchangeable) ppm. Anal.Calcd for $C_{20}H_{15}N_5O_3S$: C, 59.25; H, 3.73; N, 17.27. Found: C, 59.47; H, 3.71; N, 17.43.

General procedure for the synthesis of (III_{a-g}):

An equimolar Mixture of I_{a-g} (0.016 mol) and sulphamethoxazole (0.016 mol) was heated under reflux in acetic acid (20 ml) in presence of fused anhydrous Sodium acetate (2.44 mmol,0.2gm) for 6 h. The solid formed after cooling was filtered, washed with acetic acid, dried and crystallized from acetic acid.

4-(4-Benzylidene-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)-*N*-(3-methylisoxazol-5-yl) benzene sulfonamide (III_a) :

Mp 239°C, yield: 49 % , IR (KBr) cm^{-1}: 3379 (NH), 3109 (CH aromatic), 2974 (CH aliphatic), 1678 (C=O), 1332, 1093 (SO_2). ^1H NMR (DMSO) δ: 2.87 (s, 3H, CH_3),6.03(s, 1H, Isoxazole, C4),6.09 (m, 5H, Phenyl), 6.53(d,*j=5.22* 2H, Phenyl), 6.93 (s, 1H, =CH), 7.71(d, *j=5.73* ,2H, Phenyl), 8.15 (t, 1H, Pyridine C5), 8.24(m, 3H, Pyridine C 2, 4, 6), 10.33 (s, 1H, NH, D_2O exchangeable) ppm. Anal.Calcd for $C_{25}H_{19}N_5O_4S$: C, 61.85; H, 3.94; N, 14.42. Found: C, 62.03; H,3.98; N, 14.59.

4-(4-(4-Chlorobenzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)-*N*-(3-methylisoxazol-5-yl) benzenesulfonamide (III_b) :

Mp 243°C, yield: 52 %, IR (KBr) cm^{-1}: 3282 (NH), 3109 (CH aromatic), 2970 (CH aliphatic), 1674 (C=O), 1332, 1095 (SO_2). ^1H NMR (DMSO) δ: 2.47 (s, 3H, CH_3), 6.08 (s, 1H, Isoxazole, C4),7.72 (d, *j=5.16*, 2H, Phenyl),7.75 (s, 1H, =CH), 7.84 (d, *j=5.35*,2H, Phenyl),8.60(d, *j=5.25*,2H, phenyl), 8.93(d, *j=5.28*,2H, phenyl),9.10 (t, 1H, Pyridine C5), 9.13 (d, *j=8.61*, 2H, Pyridine C4, 6), 9.16 (s, 1H, Pyridine C2), 10.61 (s, 1H, NH, D_2O

exchangeable) ppm. Anal.Calcd for $C_{25}H_{18}Cl\ N_5O_4S$: C, 57.75; H, 3.49; N, 13.47. Found: C, 57.94; H,3.52; N, 13.54.

4-(4-(4-Hydroxybenzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)-*N*-(3-methylisoxazol-5-yl) benzenesulfonamide (III_c) :

Mp 255°C, yield: 65 % , IR (KBr) cm^{-1}: 3290 (OH), 3284 (NH), 3109 (CH aromatic), 2972 (CH aliphatic), 1674 (C=O), 1332, 1095 (SO_2). ^{1}H NMR (DMSO) δ: 2.46 (s, 3H, CH_3), 4.64 (s, H, OH, D_2O exchangeable), 6.08 (s,1H, Isoxazole, C4),7.71 (d, *j=8.61*, 2H, Phenyl), 7.73 (s, 1H, =CH), 7.86 (d, *j=5.16*, 2H, Phenyl), 8.61 (d, *j=5.16*, 2H, Phenyl), 8.62 (d, *j=5.16*, 2H, Phenyl), 8.94 (t, 1H, Pyridine C5), 9.16 (m, 3H, Pyridine C2,4, 6), 10.53 (s, H, NH, D_2O exchangeable) ppm. Anal.Calcd for $C_{25}H_{19}N_5O_5S$: C, 59.87; H, 3.82; N, 13.96. Found: C, 59.96; H, 3.91; N, 14.07.

4-(4-(4-Methoxybenzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)-*N*-(3-methylisoxazol-5-yl) benzenesulfonamide (III_d) :

Mp 245°C, yield: 63 %, IR (KBr) cm^{-1}: 3282 (NH), 3056 (CH aromatic), 2974(CH aliphatic), 1674 (C=O), 1332, 1095 (SO_2). ^{1}H NMR (DMSO) δ: 2.46 (s, 3H, CH_3), 3.80 (s, 3H ,OCH_3), 6.10 (s, 1H, Isoxazole, C4), 7.72 (d ,j=5.16, 2H, Phenyl), 7.74 (d ,j=5.16, 2H, Phenyl), 7.75 (s, 1H, =CH),7.77 (d ,*j=5.16*, 2H, Phenyl), 8.66 (d ,*j=5.16*, 2H, Phenyl), 8.97 (t, 1H, Pyridine C5), 9.16 (m ,3H, Pyridine C2,4, 6), 10.61 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO) δ: 24.54 (CH_3), 40.53 (CH_3O), 95.83 (Isoxazole C=C-O),126.18 (CH=C),128.42 (CH=C), 128.92 (Phenyl C3,5,6), 133.24 (Phenyl C2`,3`,4`,5`,6`), 142.06 ( Phenyl C 1`,1,2), 144.11 (imidazole C=N), 148.78 (Isoxazole C=N), 149.32 (Pyridine C3,4,5), 158.03 (Pyridine C2,6), 165.2 (Phenyl C4), 169.65 (Isoxazole C=C-O), 170.65(C=O). Anal.Calcd for $C_{26}H_{21}N_5O_5S$: C,60.57; H, 4.11; N, 13.58. Found: C, 60.72; H, 4.19; N, 13.72.

4-(4-(4-(Dimethylamino)benzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)-*N*-(3-methylisoxazol-5-yl)benzenesulfonamide (III_e) :

Mp 251°C, yield: 35 % , IR (KBr) cm^{-1}: 3205 (NH), 3093 (CH aromatic), 2995 (CH aliphatic), 1716 (C=O), 1334, 1091 (SO_2). ^{1}H NMR (DMSO) δ: 2.46 (s, 3H, CH_3),2.49 (m,6H, $2CH_3$) 6.08 (s,1H, Isoxazole, C4),7.77 (d, *j=5.12*, 2H, Phenyl), 7.88 (d, *j=5.14*, 2H, Phenyl), 7.90 (s, 1H, =CH),8.63 (d, *j=4.59*, 2H, Phenyl), 8.95 (d, *j=2.88*, 2H, Phenyl), 8.96 (t, 1H, Pyridine C5), 9.14 (m, 3H, Pyridine C2,4, 6), 10.75 (s, 1H, NH, D_2O exchangeable) ppm. Anal.Calcd for $C_{27}H_{24}N_6O_4S$: C, 61.35; H, 4.58; N, 15.9. Found: C, 61.54; H, 4.66; N, 16.13.

4-(4-(2-Hydroxybenzylidene)-5-oxo-2-(pyridin-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl)-*N*-(3-methylisoxazol-5-yl)benzenesulfonamide (III_f) :

Mp 246°C, yield: 33 %, IR (KBr) cm^{-1}: 3300. (NH), 3259 (OH), 3093 (CH aromatic), 2993 (CH aliphatic), 1681 (C=O), 1315, 1091 (SO_2). ^{1}H NMR (DMSO) δ: 2.29 (s, 3H, CH_3),3.33 (s, 1H, OH, D_2O exchangeable), 6.12 (s, 1H, Isoxazole),7.74 (m, 4H, Phenyl), 7.76 (s, 1H, =CH),7.78 (m, 4H, Phenyl), 7.80 (m, 3H, Pyridine C4,5,6), 10.35 (s, 1H, Pyridine C2), 11.30 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO) δ: 24.01 (CH_3), 95.84 (Isoxazole C=C-O),125.38 (CH=C),128.14 (CH=C), 128.41 (Phenyl C3,5,6), 133.24 (Phenyl C2`,3`,4`,5`,6`), 140.13 ( Phenyl C 1`,1,4), 144.14 (imidazole C=N), 148.36 (Isoxazole C=N), 151.11(Pyridine C3,4,5), 158.04 (Pyridine C2,6), 165.8 (Phenyl C2), 169.68 (Isoxazole C=C-O), 170.64 (C=O). Anal.Calcd for $C_{25}H_{19}N_5O_5S$: C, 59.87; H, 3.82; N, 13.96. Found: C, 60.04; H, 3.87; N, 14.11.

***N*-(3-Methylisoxazol-5-yl)-4-(5-oxo-2-(pyridin-3-yl)-4-(pyridin-3-ylmethylene)-4,5-dihydro-1*H*-imidazol-1-yl)benzenesulfonamide (III_g) :**

Mp 241°C, yield: 30 %, IR (KBr) cm^{-1}: 3293. (NH), 3093 (CH aromatic), 3064(CH aliphatic), 1681 (C=O), 1315, 1091 (SO_2). ^{1}H NMR (DMSO) δ: 2.47 (s, 3H, CH_3), 6.12 (s, 1H, Isoxazole), 7.74 (s, 1H, =CH), 7.76 (m, 4H, Phenyl), 7.79 (m, 6H, Pyridine C4, 4\, 5, 5\, 6.6\), 10.35 (s, 2H, Pyridine C2, 2\), 11.30 (s, H, NH, D_2O exchangeable) ppm. Anal.Calcd for $C_{24}H_{18}N_6O_4S$: C, 59.25; H, 3.73; N, 17.27. Found: C, 59.51; H, 3.70; N, 17.49.

General procedure for the synthesis of (IV_{a-g}):

A mixture of **Ia-g** (0.01 mol) and 2-aminopyridine (0.01 mol, 0.94 gm) and fused sodium acetate (6.09 mmol,0.5 gm) in glacial acetic acid (40 ml) was refluxed for 5 h. The reaction mixture was then cooled filtered, dried and crystallized from acetic acid.

4-Benzylidene-1-(pyridin-2-yl)-2-(pyridin-3-yl)-1*H*-imidazol-5(4*H*)-one (IV_a):

Mp 243°C, yield: 58 % , IR (KBr) cm^{-1}: 3149, 3120 (CH aromatic), 2927(CH aliphatic), 1716 (C=O). ^{1}H NMR (DMSO) δ: 6.85(m, 5H, Phenyl), 7.92 (s, 1H, =CH), 8.65 (m, 4H, Pyridine C4,6 3`6`), 8.96(m, 3H, Pyridine C 5, 4\, 5\), 9.14 (s, 1H, Pyridine C2) ppm. MS: m/z (% abundance) 325 (M+, 0.09%), 121.96($C_6H_4N_2O$, 100%). Anal.Calcd for $C_{20}H_{14}N_4O$: C, 73.61; H, 4.32; N, 17.17. Found: C, 73.88; H, 4.39; N, 17.39.

4-(4-Chlorobenzylidene)-1-(pyridin-2-yl)-2-(pyridin-3-yl)-1*H*-imidazol-5(4*H*)-one (IV_b):

Mp 230°C, yield: 57 %, IR (KBr) cm^{-1}: 3147, 3109 (CH aromatic), 2899(CH aliphatic), 1716(C=O). ^{1}H NMR (DMSO) δ: 7.85 (s, 1H, =CH), 8.31 (d, *j=1.6*, 2H, Phenyl), 8.34 (d, *j=1.96*, 2H, Phenyl), 8.58 (t, 1H, Pyridine C4\), 8.61(t, 2H, Pyridine C5,5`), 8.94(m, 1H, Pyridine C3`), 8.96 (m, 1H, Pyridine C4), 9.0(m, 2H, Pyridine C 6, 6\), 9.23 (s, 1H, Pyridine C2) ppm. ^{13}C NMR (DMSO) δ: 126.13 (CH=C),128.48 (CH=C), 128.82 (Phenyl C2,3,5,6), 131.04 (Phenyl C 1,4), 145.67 (Pyridine C ,3`,4`,5`), 148.64(Pyridine C3,4,5,6), 163.27 (Pyridine C2,2`,6`), 165.20 (imidazole C=N), 172.38 (C=O). Anal.Calcd for $C_{20}H_{13}ClN_4O$: C, 66.58; H, 3.63; N, 15.53. Found: C, 66.72; H, 3.63; N, 15.74.

4-(4-Hydroxybenzylidene)-1-(pyridin-2-yl)-2-(pyridin-3-yl)-1*H*-imidazol-5(4*H*)-one (IV_c):

Mp 248°C, yield: 70 %, IR (KBr) cm^{-1}: 3410 (OH), 3149, 3120 (CH aromatic), 3024(CH aliphatic), 1716 (C=O). ^{1}H NMR (DMSO) δ: 3.6 (s, 1H, OH, D_2O exchangeable), 7.89(s, 1H, =CH), 7.93 (d, *j=5.76*, 2H, Phenyl), 7.95 (d, *j=5.48*, 2H, Phenyl), 8.7 (m, 3H, Pyridine C5,4`,5`), 8.99(m, 2H, Pyridine C 4,3`), 9.009 (m, 2H, Pyridine C 6,6`), 9.17 (s, 1H, Pyridine C2) ppm. Anal.Calcd for $C_{20}H_{14}N_4O2$: C, 70.17; H, 4.12; N, 16.37. Found: C, 70.24; H, 4.17; N, 16.53.

4-(4-Methoxybenzylidene)-1-(pyridin-2-yl)-2-(pyridin-3-yl)-1*H*-imidazol-5(4*H*)-one (IV_d):

Mp 234°C, yield: 50 %, IR (KBr) cm^{-1}: 3149, 3120 (CH aromatic), 3024(CH aliphatic), 1716(C=O). ^{1}H NMR (DMSO) δ: 2.48 (s, 3H, CH_3), 7.1 (s, 1H, =CH), 7.99 (m, 2H, Phenyl), 8.08 (m, 2H, Phenyl),), 8.75 (m, 3H, Pyridine C5,4`,5`), 8.77(m, 2H, Pyridine C 4,3`), 9.02 (m, 2H, Pyridine C 6,6`), 9.16 (s, 1H, Pyridine C2) ppm. ^{13}C NMR (DMSO) δ: 39.77 (OCH_3), 126.76 (CH=C),129.42 (CH=C), 148.13 (Phenyl C2,3,5,6), 145.88 (Phenyl C 1,4), 148.1 (Pyridine C ,3`,4`,5`), 148.39(Pyridine C2,2`3,4,5,6,6`), 164.72 (imidazole C=N), 164.95 (C=O). MS: m/z (% abundance) 356 (M^+, 1.33%), 57 (C_2H_3NO, 100%). Anal.Calcd for $C_{21}H_{16}N_4O_2$: C, 70.77; H, 4.53; N, 15.72. Found: C, 70.93; H, 4.61; N, 15.89.

4-(4-(Dimethylamino)benzylidene)-1-(pyridin-2-yl)-2-(pyridin-3-yl)-1*H*-imidazol-5(4*H*)-one (IV_e):

Mp 245°C, yield: 70%, IR (KBr) cm^{-1}: 3149, 3120 (CH aromatic), 3024(CH aliphatic), 1716(C=O). ^{1}H NMR (DMSO) δ: 2.6 (s, 6H, $2CH_3$), 7.86 (s, 1H, =CH), 7.88 (m, 2H, Phenyl), 7.90 (m, 2H, Phenyl), 8.62 (m, 3H, Pyridine C5,4`,5`), 8.64 (m, 2H, Pyridine C 4,3`), 8.96 (m, 2H, Pyridine C 6,6`), 9.16 (s, 1H, Pyridine C2) ppm. Anal.Calcd for $C_{22}H_{14}N_5O$: C, 71.53; H, 5.18; N, 18.96. Found: C, 71.67; H, 5.22; N, 19.10.

4-(2-Hydroxybenzylidene)-1-(pyridin-2-yl)-2-(pyridin-3-yl)-1*H*-imidazol-5(4*H*)-one (IV_f):

Mp 250°C, yield: 40 %, IR (KBr) cm^{-1}: 3320 (OH), 3149, 3120 (CH aromatic), 3024(CH aliphatic), 1716 (C=O). ^{1}H NMR (DMSO) δ: 3.95 (s, 1H, OH, D_2O exchangeable), 7.05 (s, 1H, =CH), 7.95 (m, 2H, Phenyl), 7.96 (m, 2H, Phenyl), 8.69 (t, 1H, Pyridine C4\), 8.7(t, 2H, Pyridine C5,5`), 8.98(m, 1H, Pyridine C3`), 8.91 (m, 1H, Pyridine C4), 9.13(m, 2H, Pyridine C 6, 6\), 9.17 (s, 1H, Pyridine C2) ppm. MS: m/z (% abundance) 342 (M^+, 1%), 172 ($C_9H_6N_3O$, 100%). Anal.Calcd for $C_{20}H_{14}N_4O_2$: C, 70.17; H, 4.12; N, 16.37. Found: C, 70.38; H, 4.11; N, 16.49.

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1-(Pyridin-2-yl)-2-(pyridin-3-yl)-4-(pyridin-3-ylmethylene)-1*H*-imidazol-5(4*H*)-one (IV_g):

Mp 238°C, yield: 30 %, IR (KBr) cm^{-1}: 3154 (CH aromatic), 3100(CH aliphatic), 1724 (C=O). ^1H NMR (DMSO) δ: 7.83 (s, 1H, =CH), 7.85 (m, 4H, Pyridine C 5,3`,5`,5``), 7.87 (m, 3H, Pyridine C 4,4`,4``), 8.59 (m, 2H, Pyridine C 6`,6``), 8.95 (m, 2H, Pyridine C6, 2``) 9.17 (s, 1H, Pyridine C2) ppm. Anal.Calcd for $C_{19}H_{13}N_5O$: C, 69.71; H, 4.00; N, 21.39. Found: C, 69.87; H, 3.98; N, 21.52.

Biological evaluation

The in vitro COX-1 and COX-2 inhibition by compounds **IIa-g, IIIa-g and IVa-g** was evaluated through Cayman's colorimetric COX (ovine) inhibitor screening assay measures the Peroxidase component of COX 1 & 2. The Peroxidase activity is assayed calorimetrically by monitoring the appearance of oxidized N,N,N`,N` -tetra methyl -p-phenylenediamine (TMPD) at 590 nm. The colorimetric COX (ovine) inhibitor screening assay kit includes both ovine COX-1 and COX-2 enzymes in order to screen isozyme-specific inhibitors.

Pre-Assay Preparation:

Dilute 3 mL of assay buffer concentrate with 27 mL of HPLC - grade water. This final assay buffer (0.1 M Tris-HCl, pH 8) should be used for dilution of heme and COX enzymes prior to assaying. This vial contains a solution of heme in dimethylsulphoxide. Dilute 88 µL of heme with 1.912 mL of diluted assay buffer prior to use. A vial contains a solution of ovine COX-1 and should be kept on ice when thawed. Dilute 200 µL of enzyme with 400 µL of diluted assay buffer and store on ice. A vial contains a solution of ovine COX-2 and should be kept on ice when thawed. Dilute 200 µL of enzyme with 400 µL of diluted assay buffer and store on ice. A vial contains a solution or arachidonic acid in ethanol. Transfer 100 µL of the supplied substrate to another vial, add 100 µL of potassium hydroxide (item no. 760115), vortex, and dilute with 1.8 mL of HPLC - grade water to achieve a final concentration of 1.1 mM. Use the prepared arachidonic acid solution within 30 minutes. A 20 µL aliquot will yield a final concentration of 100 µM in the wells. A vial contains 0.1 M potassium hydroxide (KOH). A vial contains a solution of TMPD.

Performing the Assay

Background wells: add 160 µL of assay buffer, and 10 µL of heme to three wells.

100% Initial Activity wells: add 150 µL assay buffer, 10 µL of heme, and 10 µL of enzyme (either COX-1 or COX-2) to three wells. Inhibitor wells: add 150 µL of assay buffer, 10 µL of heme, and 10 µL of enzyme (either COX-1 or COX-2) to three wells. Add (10, 5 , 2.5mg) of inhibitor to the inhibitor wells and 10 µL of solvent (methanol, dimethylsulphoxide or ethanol) to the 100% Initial Activity wells and background wells, this process was repeated three times. Carefully shake the plate for a few seconds and incubate for five minutes at 25°C. Add 20 µL of the colorimetric substrate solution to all the wells that you are using. Add 20 µL of arachidonic acid to all the wells you are using. Carefully shake the plate for few seconds and incubate for five minutes at 25°C. Read the absorbance at 590 nm using a plate reader.

Data Analysis

Determine the average absorbance of all the samples. Subtract the absorbance of the background wells from the Initial Activity sample, then divide by the 100% Initial Activity sample, and multiply by 100 to give the percent inhibition. Graph the percent inhibition and determine the IC_{50} value by using the three results obtained.

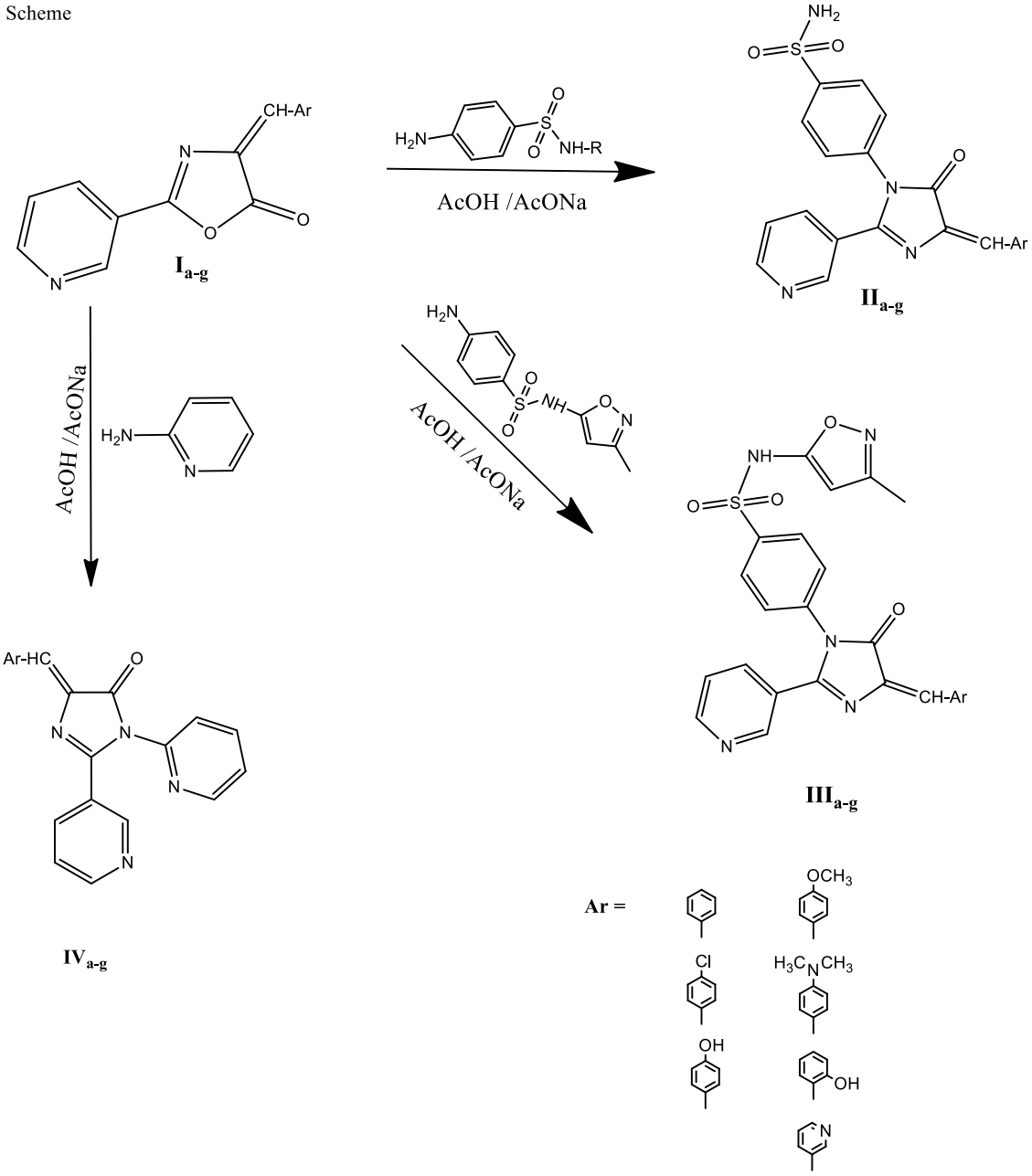
RESULTS AND DISCUSSION

Chemistry

Literature survey revealed that the modification of oxazolone ring system to imidazolone ring system was carried out through the aminolysis of the oxazolone ring with primary amines resulting in the formation of 2-imidazolin-5-one. [11] So, the target compounds **IIa-g, IIIa-g** and **IVa-g**were synthesized according to the reaction sequence outlined in scheme 1 starting with oxazolone derivatives **Ia-g** produced from the reported

procedure.[10] Followed by reaction of **Ia-g** in a three way synthesis by the primary amino group of sulphanilamide, sulphamethoxazole and 2-amino pyridine in acetic acid and in the presence of sodium acetate. The produced derivatives` structure was confirmed.IR spectral bands showed the appearance of absorption bands at range of3379-3205 cm^{-1} corresponding to NH as well as the appearance of distinctive bands to SO_2 at 1315, 1091 cm^{-1} for compounds **IIa-g and IIIa-g**. Additionally, the 1HNMR of **IIa-g and IIIa-g** showed appearance of singlet signal equivalent to NH protons at range from 10.33 to 11.30 ppm. In addition, compounds **IIIa-g** have singlet signal of protons of CH_3 at 2.29 to 2.87 ppm and characteristic singlet signal of proton of CH of isoxazole moiety at 6.03 to 6.12 ppm of sulphamethoxazole added group.13C NMR showed distinctive signals of added aromatic carbon of sulphanilamide and sulphamethoxazole. Moreover, **IVa-g**spectral data showed the appearance of absorption band at range of 1716-1645 cm-1 corresponding to CO. Additionally, the 1HNMR of VIa-g compounds showed appearance of distinctive singlet signal of (=CH) at range 7.50-7.90 ppm & multiple signal of hydrogens of the two pyridine rings at range 8.60-9.0 ppm. Additionally, Compound IVa mass spectrum showed molecular ion peak at 325 (M^+, 0.09%). Compound IVb 13CNMR showed the distinctive signal of (=CH) carbon at 126.13 ppm and (C=N) of imidazole moiety at 165.20 ppm.

Scheme



I_{a-g} II_{a-g} III_{a-g} IV_{a-g}

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Biological evaluation

Compounds **IIa-g,IIIa-g and IVa-g** were evaluated for their inhibitory activities towards COX-1 and COX-2 enzymes (Tables 1,2 and 3) **Fig 2**

Table1: The IC_{50} values of celecoxib, diclofenac sodium and IIa-g against COX-1 and COX-2 enzymes at different concentrations (10, 5, 2.5 mg each)

Compd.	IC50 COX1 umol	IC50 COX2 umol
Celecoxib	14.8	0.05
diclofenac sodium	3.9	0.8
IIa	3.11	**0.12**
IIb	6.41	**0.37**
IIc	8.44	0.97
IId	4.62	**0.37**
IIe	5.27	0.62
IIf	9.74	1.12
IIg	11.23	1.42

Table2:The IC_{50} values of celecoxib , diclofenac sodium and IIIa-g against COX-1 and COX-2 enzymes at different concentrations (10, 5, 2.5 mg each)

Compd.	IC50 COX1 umol	IC50 COX2 umol
Celecoxib	14.8	0.05
diclofenac sodium	3.9	0.8
IIIa	7.24	0.97
IIIb	4.61	**0.33**
IIIc	10.22	1.52
IIId	6.59	0.88
IIIe	8.52	1.04
IIIf	12.61	1.75
IIIg	9.65	1.34

Table 3: The IC_{50} values of celecoxib , diclofenac sodium and IVa-g against COX-1 and COX-2 enzymes at different concentrations (10, 5, 2.5 mg each)

Compd.	IC50 COX1 umol	IC50 COX2 umol
Celecoxib	14.8	0.05
diclofenac sodium	3.9	0.8
IV_a	4.62	**0.41**
IV_b	3.56	**0.19**
IV_c	8.42	1.09
IV_d	6.52	0.78
IV_e	12.51	1.96
IV_f	10.23	1.56
IV_h	11.62	2.11

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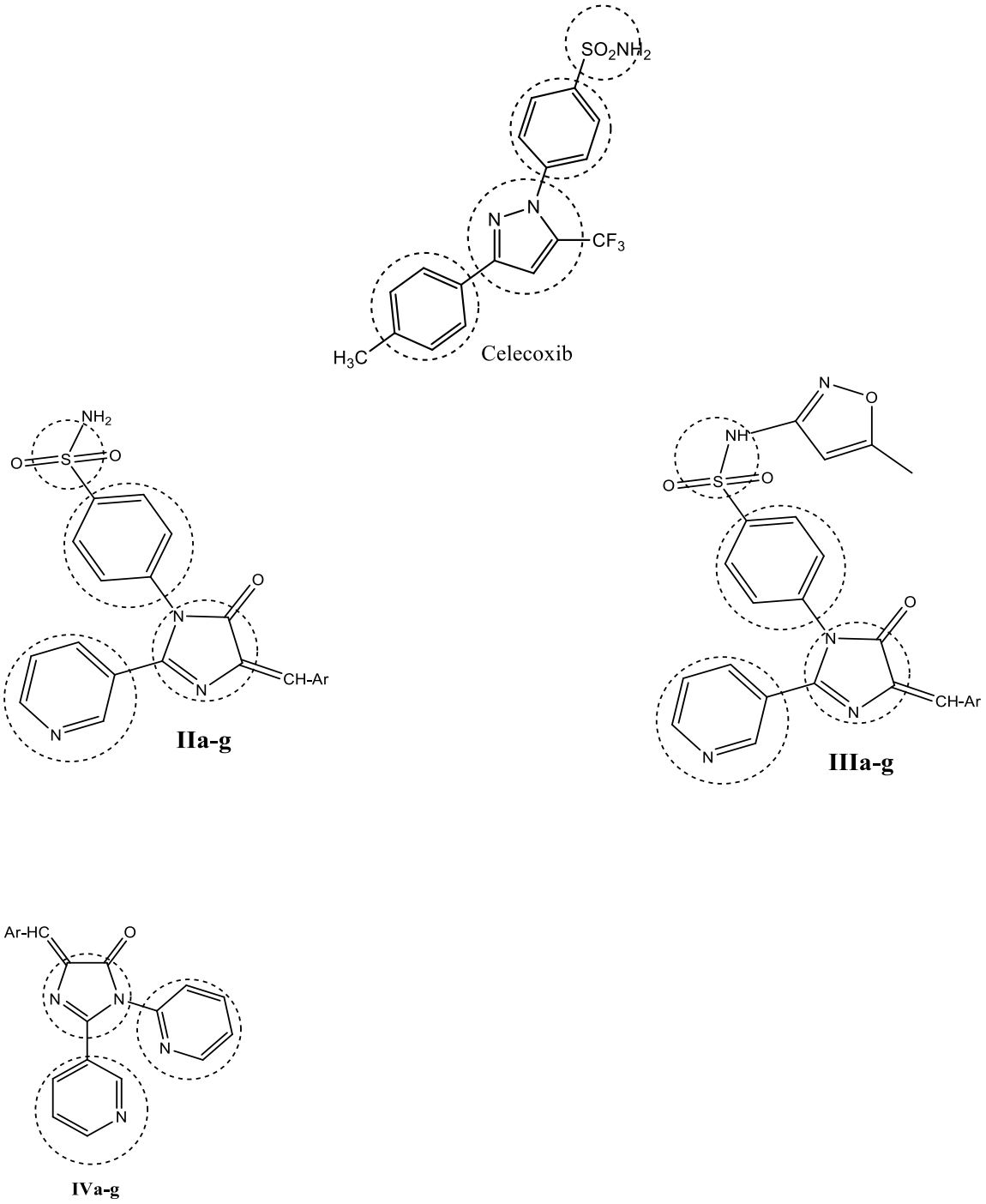
Fig 2: Structure resemblance of new derivatives IIa-g, IIIa-g IVa-g to celecoxib



The new derivatives were substituted by an aldehyde group in addition to sulphanilamide, Sulfamethoxazole groups and pyridine ring, additional rings contributing to the large size of COX-2 inhibitors. Since COX-2 selective drugs have bulky structure which makes the molecules too large to fit into the COX-1 active site but still able to fit the COX-2 active site. [12]Moreover, it was reported that COX-2 inhibiting activityis achieved by tricyclic structure of inhibitors bearing unsaturated heterocyclic ring with attached two aromatic rings. [13]Furthermore, substituted benzene sulfonamide group play a crucial role for COX-2 inhibitory activity. [14]The new derivatives showed good to moderate inhibitory activity against COX-2 enzyme close to that of celecoxib and better than NSAID drug diclofenac. The better activity is due to the bulkier size which make it fit better to COX-2 enzyme, also due the addedbenzene sulfonamide group and pyridine ring. The most potent derivative was **IIa** and **IVb** with IC50 value of 0.12 and 0.19 µM which contributes to the

features needed for COX-2 activity. Although compounds **IIIb, IIb and IId and IVa** show less inhibitory activity but still close to celecoxib and better than Diclofenac with IC_{50} values of 0.33, 0.37, 0.37 and 0.41 µM. On the other hand, **IIe, IVd, IIId, IIc and IIIa** showed moderate inhibitory activity. The least activity was observed **by IIf ,IIg, IIIc, IIIe, IIIf,IIIg, IVc, IVe , IVf and IVg** but still inhibit COX-2 enzyme better than COX-1 enzyme.

From the above and upon examining different groups for Cox-2 inhibitory activity the bulkier groups and derivatives bearing benzene sulfonamide group were found more potent and more selective as COX-2 inhibitors.

CONCLUSION

New imidaolone derivatives were synthesized bearing different substitutions to elaborate the groups that better inhibit COX-2 enzyme over COX-1 enzyme, different concentrations were used (10,5 ,2.5mg) to measure their IC_{50} activity and compare it to standard drugs. The most potent derivative was **IIa**with substituted benzene sulfonamide groupin addition to its tricyclic structure necessary for COX-2 inhibitory activity. Also compound **IVb** was found to be potent as COX-2 more than COX-1 with its additional pyridine ring and *p*-chloro substitution. Moderate activity was demonstrated by **IIIb, IIb and IId** proving that the benzene sulfonamide group is necessary for activity in addition to the *p*-chloro and *p*-methoxy substitution.

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