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SYNTHESIS AND SCREENING OF 2-HYDROXY-N-(2,4¹-DIOXOSPIRO [INDOLINE-3,2¹-THIAZOLIDIN]-3¹-YL) BENZAMIDES FOR ANTIINFLAMMATORY ACTIVITY

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ABSTRACT

A novel synthesis of 2-hydroxy-N-(2,4¹-dioxospiro[indoline-3,2¹-thiazolidin]-3¹-yl) benzamide derivatives was synthesized by cyclization of isatin hydazones with thioglycollic acid. The synthesized compounds were characterized by spectral data (IR, ¹HNMR, MASS) and evaluated for In-vitro, In-vivo anti-inflammatory activity. The test compounds exhibited significant potency to inhibit COX-2 enzyme values between46.23±0.38to73.24±0.35, Among the tested compounds VIi(R=5,6-dichloro), VI f (R=5-F), VI b (R=5-Cl), are considered to possess more potent anti-inflammatory activity when compared to standard drug indomethacin. By in-vitro anti-inflammatory activity the compound VIi (R=5,6-dichloro) VI f (R=5-F), VI b (R=5=Cl) and VIH(R=5-Br) are considered to possess more potent anti-inflammatory activity when compared to standard drug indomethacin.

KEY WORDS

lisatin, Thiazole, Inflammation, Cyclooxygenase

INTRODUCTION:

Cyclooxygenases (COX) or prostaglandin endoperoxide synthases are the key enzymes in the synthesis of prostaglandins, the main mediators of inflammation, pain, and increased body temperature (hyperpyrexia). The body produces two main isoforms of COX proteins, that is, cyclooxygenases- 1 (COX-1) and cyclooxygenases-2 (COX-2). The COX-1 is responsible for formation of important biological mediators such as prostanoids, including prostaglandins, prostacyclin, and thromboxane, and involved in pain causing, blood clotting, and protecting the stomach [1], whereas COX-2 is involved in the pain by inflammation and plays a major role in prostaglandin biosynthesis in inflammatory cells and central nervous system [2]. When COX-1 is inhibited, inflammation is reduced, but the protection of the lining of the stomach is also lost. This can cause stomach upset as well as ulceration and bleeding from the stomach and even the intestines. Whereas COX-2 is usually specific to inflamed tissue, there is much less gastric irritation associated with COX-2 inhibition together with the decreased risk of peptic ulceration [3]. Therefore, selective COX-2 inhibitors such as celecoxib and rofecoxib had been developed for ease of inflammation associated with COX [4]. The use of coxib drugs such as rofecoxib (VioxxW) and valdecoxib (BextraW) was withdrawn from the market in 2004 and 2005, respectively, because of increased risk of heart attacks and strokes with long-term use [5]. At present, celecoxib (CelebrexW) is the only COX-2 inhibitor available in the United States. Hence, there is a need for COX-2 inhibitor with no adverse effects.

It is evident from literature that isatin derivatives are known to be associated with broad spectrum of biological activity like antibacterial (1), anti-

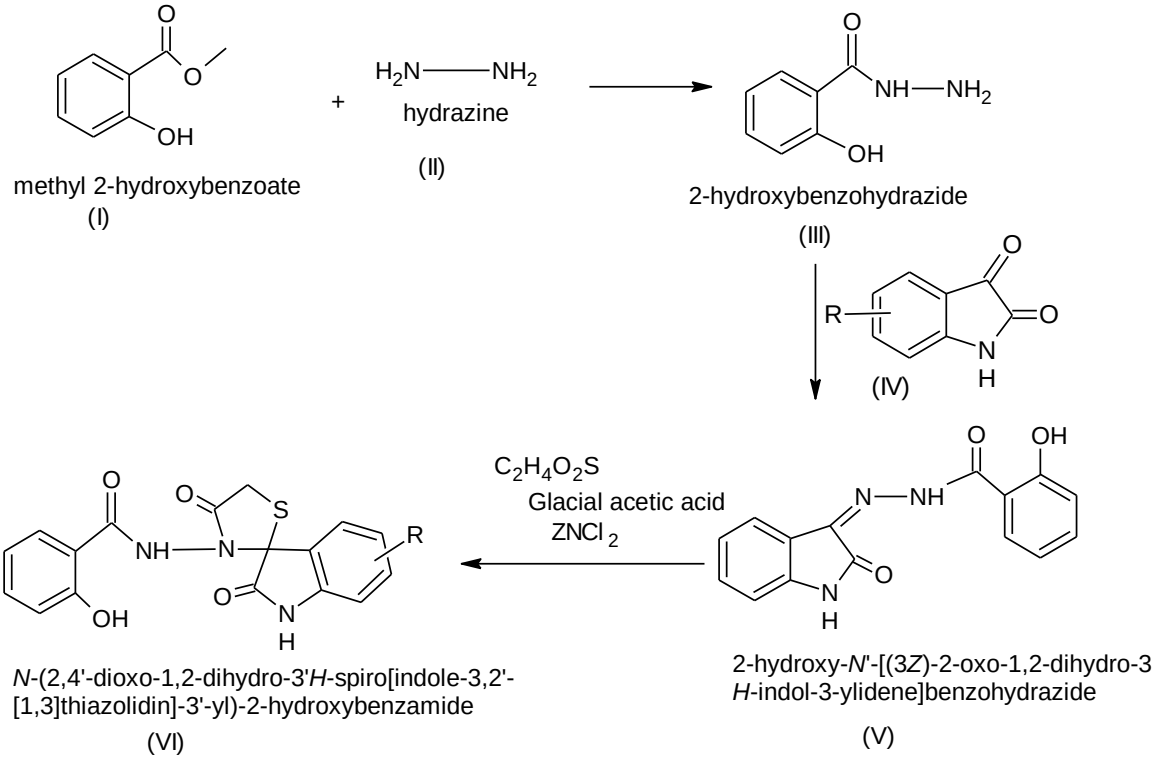
inflammatory (2), analgesic (3), anti-viral (4), anti-fungal (5), anti-tubercular (6), anti-depressant (7). Isatin hydrazones have been reported to possess anticonvulsant (7) activity also. In view of these facts and as a continuation of our work in the laboratory, prompted us to synthesize some new N-(2,4'-dioxo-1,2-dihydro-3'*H*-spiro[indole-3,2'-[1,3] thiazolidin]-3'-yl)-2-hydroxybenzamide All the synthesized compounds were screened for their anti-inflammatory activity.

It has been reported that the nature of substituents at the 2- or 3-position of the indole nucleus plays an important role in modulating their anti-inflammatory properties [14–18]. Amide-containing compounds have been shown to possess a wide range of biological activities, including anti-inflammatory properties. Interestingly, the replacement of the carboxylic groups by amide groups in NSAID drugs indomethacin, meclofenamic acid, and ketoprofen conferred the compounds greater selectivity for COX-2 over the COX-1 enzyme [19]. Literature survey reveals that 4-hydroxybenzohydrazide ring is important for antimycobacterial activity [20]. In addition, many thiazoline derivatives exhibit a wide variety of biological activities such as antimicrobial [21], anti-inflammatory [22], antihistaminic [23], antihypertensive [24], hypnotic [25], and anticonvulsant [26]. Keeping in view of biological importance of the two molecular moieties, namely, isatins and 2-hydroxybenzohydrazide, to study the condensation of isatins with 2-hydroxybenzohydrazide (**II**) has been felt worthwhile as depicted in Scheme 2. The synthesized compounds were screened for *in vivo* anti-inflammatory activity.

2. MATERIALS AND METHODS:

All the chemicals used were of analytical grade and obtained from Himedia and SD Fine, melting points were determined by open capillary tubes using VEEGO VMP-D Digital melting point. FTIR spectra of the powdered compounds were recorded using KBr on a JASCO FTIR 4100 series and are reported in cm_1 and 1H NMR spectra were recorded on a Varian Mercury YH300 (300 MHz FT NMR) spectrophotometer using TMS as an internal reference (Chemical shift represented in ppm). Purity of the compounds was checked on TLC plates using silica gel G as stationary phase and iodine vapors as the visualizing agent.

3. CHEMISTRY:



Scheme-1

3.1. Synthesis of Indole-2,3-diones

*3.1.1. Isonitrosoacetanilides (**I1**).* In a 5 lit. RB flask, chloral hydrate (0.54 mol) and 1200mL of water were placed. To this solution, crystallized sodium sulphate (1300 g) was then added followed by a solution of an appropriate aromatic amine (**I**) in 300mL of water and concentrated hydrochloric acid (0.52mol). Finally, a solution of hydroxylamine HCl (1.58mol) in 500mL of water was added. The contents of the flask were heated over a wire-gauge by a Mecker burner so that vigorous boiling begins in about 45minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilides started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried, and purified by recrystallization from suitable solvent(s).

3.1.2. Indole-2,3-diones. Sulphuric acid (600 g, d, 1.84,326 mL) were warmed at 50^{0}C in a one-liter RB flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide (**II**, 0.46mol) was added at such a rate so as to maintain the temperature between 60^{0}C and 70^{0}C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80^{0}C and maintained at that temperature for 10 minutes, to complete the reaction. Then the reaction mixture was cooled to room temperature and poured on crushed ice (2.5 kg) while stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water, and dried. Purification of the compound was affected by the recrystallization from methanol [27].

3.2. Preparation of 2-Hydroxybenzohydrazide *(III).* In a 500mL of RB flask, 10 g of methyl salicylate (**I**) and 50mL of distilled alcohol were placed and the reaction mixture was shaken for 5 minutes. To this add 20mL of hydrazine hydrate **(II)** (99%) and the contents of the flask were refluxed for 3 hours, the completion of the reaction monitored by TLC. The resultant white crystalline solid was filtered and washed repeatedly, with small portions of cold alcohol. The product was dried and purified by recrystallization from methanol, yield 90%, m.p. 251–254^{0}C

3.3. Synthesis of 2-Hydroxy-N-(2-oxoindolin-3-ylidene) benzohydrazide(V)

A mixture of an appropriate indole-2,3- Dione **(IV).** (0.01 mol) and 2-hydroxybenzohydrazide **(III)**(0.01mol) was taken into methanol (50 mL) in presence of glacial acetic acid which was heated under reflux on water bath for 6-7 hours. The coloured compounds were thus obtained upon cooling, were filtered, were washed with small portions of water. And recrystallize by using methanol.

3.4. Synthesis of 2-hydroxy-N-(2, 4^1-dioxospiro[indoline-3, 2^1-thiazolidin]-3^1-yl) benzamide (VI). To the above compound (V) (0.01mol)add thioglycollic acid (0.01mol)and add pinch of zinc chloride and reflux in presence of glacial acetic acid for about 6-7 hrs cool the reaction mixture poured into crushed ice.Then the solution is neutralized with sodium carbonate solution and filter the solution collect the compound and dry, recrystalise with methanol.

Physical data of the newly synthesized compounds (VIIa–j) Scheme 1:

S.No.	Compound	R	Mol.F	Mol.Wt.	M.P(^{0}C)	%Yield
1	VIa	H	$C_{17}H_{13}N_3O_4S$	355	260-262	75
2	VIb	5-Cl	$C_{17}H_{12}ClN_3O_4S$	389	285-287	67.26
3	VIc	7-Cl	$C_{17}H_{12}ClN_3O_4S$	389	290-292	70
4	VId	5-CH_3	$C_{18}H_{15}N_3O_4S$	369	302-304	57
5	VIe	7-CH_3	$C_{18}H_{15}N_3O_4S$	369	270-272	62
6	VIf	5-F	$C_{18}H_{16}FN_3O_4S$	389	250-252	73.19
7	VIg	7-F	$C_{18}H_{16}FN_3O_4S$	389	198-200	66.49
8	VIh	5-Br	$C_{17}H_{12}BrN_3O_4S$	434	320-322	75
9	VIi	5,6-Dichloro	$C_{17}H_{11}Cl_2N_3O_4S$	424	310-312	60
10	VIj	5-NO_2	$C_{17}H_{12}N_4O_6S$	400	294-296	58
11	VIk	7-NO_2	$C_{17}H_{12}N_4O_6S$	400	333-335	62
12	VIl	5-OH	$C_{17}H_{13}N_3O_5S$	371	275-277	67

Spectral Data of the Synthesized Compounds:

VI(a) 2- hydroxy-N-(2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl)benzamide:IR spectrum (KBr, cm^{-1}) 3073.01(C-H aromatic), 1682.02(C=Ostr), 1620.39(C=Nstr), 1520.06(C=C aromatic), 680.02(S-CHstr); ^{1}H NMR (400MHz CDCl3, δ ppm): 3.44 (s,2H,CH_2), 6.97-6.98(s, 2H, OH),7.04-7.05(d, 1H, aromatic),7.13-7.20(m, 2H aromatic),7.14-7.46(m, 2H, aromatic),7.69-7.7(d, 1H, aromatic),8.61(s, 1H, amide);^{13}C NMR(100MHz,CDCl3):32.4, 85.8, 115.2, 117.8, 119.8, 121.4, 124.8, 127.8, 127.8, 128.9, 129.8, 133.5, 141.1, 159.4, 164.8, 168.2, 168.8: MS: m/z:355.06 (100.0%), 356.07(18.7%)**.** Elemental analysis:$C_{17}H_{13}N_3O_4S$ Calculated Values:C-57.46, H-3.69, N-11.82, S-9.02: Observed Values: C-57.15, H-3.52, N-11.72, S-9.01.

VI(b) 2- hydroxy-N-(5-Chloro-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl)benzamide:IR spectrum(KBr, cm^{-1}) : 3069.12(C-H aromatic), 1682.02(C=Ostr), 1620.39 (C=Nstr), 1520.06 (C=C aromatic), 680.32 (S-CHstr);); ^{1}H NMR (400MHz CDCl3, δ ppm): 3.41 (s, 2H, CH_2), 6.95-6.97 (s, 2H, aromatic OH),7.01-7.04 (d, 1H, aromatic),7.10-7.18 (m, 2H aromatic),7.38 -7.40 (m, 2H, aromatic),7.69-7.70 (d, 1H, aromatic), 8.61(s, 1H, amide) ^{13}C NMR(100MHz,CDCl3):-32.4, 85.3, 111.3, 117.8, 119.8, 121.4, 127.9, 128.9, 129.2, 129.6, 130.4, 133.5, 139.2, 159.4, 164.8, 168.2, 168.8. MS:m/z:389.02 (100.0%), 391.02 (36.7%), 390.03(18.7%), Elemental analysis: $C_{17}H_{12}ClN_3O_4S$ Calculated Values: C-52.38, H-3.10, N-10.78, S-8.23: Observed Values: C-52.26, H-3.02, N-10.65, S-8.20:

VI(c) 2- hydroxy-N-(7-Chloro-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl)benzamide: IR spectrum(KBr, cm^{-1}) : 3069.12(C-H aromatic), 1682.02(C=Ostr), 1620.39(C=Nstr), 1520.06(C=C aromatic), 680.32(S-CHstr); ^{1}H NMR (400MHz CDCl3, δ ppm): 3.38 (s, 2H, CH_2),6.92-6.95 (s, 2H, OH),7.01-7.03 (d, 1H, aromatic), 7.13-7.20 (m, 2H aromatic), 7.40-7.46(m,2H,aromatic),7.69-7.7(d,1H,aromatic),8.61(s,1H,amide); ^{13}C NMR(100MHz,CDCl3): 32.4, 85.3, 117.8, 119.8, 121.4, 126.2, 127.9, 128.9, 129.02, 192.2, 131.0, 133.5, 143.9, 159.4, 164.8, 168.2, 168.8. MS:m/z:389.02(100.0%), 391.02 (36.7%), Elemental analysis: $C_{17}H_{12}ClN_3O_4S$ Calculated Values: C-52.38, H-3.10, N-10.78, S-8.23: Observed Values: C-52.35, H-3.01, N-10.68, S-8.20:

VI(d) 2- hydroxy-N-(5-methyl-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl) benzamide

IR spectrum(KBr, cm^{-1}) 3052.12(C-H aromatic), 1678.02(C=Ostr), 1620.39(C=Nstr), 1520.06(C=C aromatic), 680.32(S-CHstr); ^{1}H NMR (400MHz CDCl3, δ ppm): 3.44 (s, 2H, CH_2), 6.97-6.98(s, 2H, aromatic OH), 7.04-7.05 (d,1H,aromatic), 7.13-7.20 (m,2H aromatic), 7.14-7.46 (m, 2H, aromatic), 7.69-7.7 (d, 1H, aromatic), 8.61 (s, 1H, amide) ^{13}C NMR(100MHz,CDCl3): -21.6, 32.4, 86.1, 115.3, 117.8, 119.8, 121.4, 127.7, 128.1, 128.9, 131.7, 133.5, 134.5, 138.1, 159.4, 164.8, 168.2, 168.8. MS:m/z: 369.08 (100.0%), 370.08 (21.7%); Elemental analysis: $C_{18}H_{15}N_3O_4S$ Calculated Values: C-58.53, H-4.09, N-11.38, S-8.68: Observed Values: C-58.51, H-4.05, N-11.33, S-8.62.

VI(e) 2- hydroxy-N-(7-methyl-2, 4^1- dioxospiro [indoline-3, 2^1-thiazolidin]-3^1-yl) benzamide:

IR spectrum(KBr, cm^{-1}) 3069.12(C-H aromatic), 1682.02(C=Ostr), 1620.39(C=Nstr), 1520.06(C=C aromatic), 680.32(S-CH.str); ^{1}H NMR (400MHz CDCl3, δ ppm): 3.44(s,2H,CH_2),6.97-6.98(s, 2H, aromaticOH),7.04-7.05(d,1H,aromatic),7.13-7.20(m,2H aromatic),7.14-7.46(m,2H,aromatic),7.69-7.7(d, 1H, aromatic),8.61(s, 1H, amide); ^{13}CNMR (100MHz,CDCl3): 17.3, 32.4, 86.1, 117.8, 119.8, 121.4, 124.7, 126.8, 127.7, 128.9, 129.6, 131.3, 133.5, 141.1, 159.4, 164.8, 168.2, 168.8. MS:m/z:369.08(100.0%),370.08(21.7%), Elemental analysis: $C_{18}H_{15}N_3O_4S$ Calculated Values: C-58.53, H-4.09, N-11.38, S-8.68: Observed Values: C-58.50, H-4.06, N-11.33, S-8.61.

VI(f) 2- hydroxy-N-(5-fluoro-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl)benzamide:IR spectrum(KBr, cm^{-1}): 3078.12(C-H aromatic), 1689.02(C=Ostr), 1625.39(C=Nstr), 1527.06(C=C,aromatic),685.32(S-CHstr);^{1}HNMR(400MHz,CDCl3,δ,ppm): 3.47(s,2H,CH_2),6.98-7.0 (s, 2H, aromatic OH), 7.04-7.05(d, 1H, aromatic), 7.15-7.26(m, 2H aromatic),7.14-7.46(m, 2H, aromatic), 7.69-7.7(d, 1H, aromatic),8.61(s, 1H, amide); ^{13}CNMR(100MHz,CDCl3): 32.4, 85.8, 111.1, 114.6, 116.8, 117.8, 119.8, 121.4, 128.9, 129.4, 133.5, 136.7, 159.0, 159.4, 164.8, 168.2, 168.8. MS:m/z: 373.05 (100.0%), 374.06(18.7%), 375.05(4.7%); Elemental analysis: $C_{18}H_{16}FN_3O_4S$ Calculated Values: C-54.69, H-3.24, N-11.25, S-8.59: Observed Values:C-54.65, H-3.22, N-11.23, S-8.52.

VI(g) 2- hydroxy-N-(7-fluoro-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl) benzamide:

IR spectrum(KBr, cm^{-1}): 3072.12(C-H aromatic), 1682.02 (C=Ostr), 1620.39 (C=Nstr), 1520.06 (C=C aromatic), 680.32 (S-CHstr); ^{1}HNMR (400MHz,CDCl3,

δ,ppm): 3.41(s, 2H, CH_2),6.97-6.98(s, 2H, aromatic OH), 7.10-7.12 (d, 1H, aromatic), 7.17-7.20 (m, 2H aromatic),7.14-7.46 (m, 2H, aromatic), 7.69-7.7 (d, 1H, aromatic), 8.61 (s, 1H,amide); ^{13}CNMR(100MHz,CDCl3): 32.4, 85.8, 114.6, 116.8, 117.8, 119.8, 121.4, 125.4, 126.4, 128.9, 129.1, 133.5, 159.4, 163.3, 164.8, 168.2, 168.8. MS:m/z:373.05(100.0%), 374.06(18.7%), 375.05(4.7%), Elemental analysis: $C_{18}H_{16}FN_3O_4S$ Calculated Values: C-54.69, H-3.24, N-11.25, S-8.59: Observed Values: C-54.64, H-3.20, N-11.23, S-8.57.

VI(h) 2- hydroxy-N-(5-Bromo-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl) benzamide:

IR spectrum(KBr, cm^{-1}) : 3069.12(C-H aromatic), 1685.02(C=Ostr), 1625.39(C=Nstr), 1520.06(C=Caromatic), 685.32(S-CHstr); ^{1}HNMR(400MHz,CDCl3,δ,ppm): 3.42(s,2H,CH_2), 6.97-6.98(s,2H,aromatic OH), 7.01-7.03(d,1H,aromatic), 7.13-7.20(m,2H aromatic), 7.14-7.46(m,2H,aromatic), 7.69-7.7(d,1H,aromatic), 8.61(s,1H,amide) ^{13}CNMR(100MHz,CDCl3): 32.4, 85.1, 117.8, 119.2, 119.8, 121.4, 124.3, 128.9, 130.0, 130.7, 133.5, 134.6, 140.1,159.4, 164.8, 168.2, 168.8. MS:m/z: 434.97 (100.0%), 432.97(98.0%), 435.97(20.02%), 433.98(18.3%), Elemental analysis: $C_{17}H_{12}BrN_3O_4S$ Calculated Values: C-47.02, H-2.79, N-9.68, S-7.38: Observed Values: C-47.0, H-2.75, N-9.62, S-7.33:

VI(i) 2- hydroxy-N-(5,6-Dichloro-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl)benzamide:

IR spectrum(KBr, cm^{-1}) : 3062.12(C-H aromatic), 1688.02(C=Ostr), 1628.39(C=Nstr), 1529.06(C=C aromatic), 681.32 (S-CHstr); ^{1}HNMR(400MHz, CDCl3, δ, ppm): 3.44(s, 2H, CH_2), 6.97-6.98(s,2H,aromatic OH), 7.04-7.05(d,1H,aromatic), 7.13-7.20(m, 2H aromatic), 7.14-7.46(m, 2H, aromatic), 7.69-7.7(d, 1H, aromatic), 8.61(s, 1H,amide) ^{13}CNMR(100MHz,CDCl3): 32.4, 85.3, 117.8, 119.8, 121.4, 123.9,127.3, 128.1, 128.9, 130.1, 131.0, 133.5, 140.6, 159.4, 164.8, 168.2, 168.8. MS:m/z: 422.98(100.0%), 424.98(68.5%), 423.99(18.7%), 426.98(13.4%); Elemental analysis: $C_{17}H_{11}Cl_2N_3O_4S$ Calculated Values: C-48.13, H-2.61, N-9.90, S-7.56: Observed Values: C-48.10, H-2.59, N-9.89, S-7.52:

VI(j) 2- hydroxy-N-(5-Nitro-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl) benzamide

IR spectrum(KBr, cm^{-1}): 3069.12(C-H aromatic), 1682.02(C=Ostr), 1620.39(C=Nstr), 1520.06(C=C aromatic), 680.32(S-CHstr); ^{1}HNMR(400MHz,CDCl3, δ, ppm): 3.44(s, 2H,CH_2), 6.97-6.98(s,2H,aromatic OH), 7.04-7.05(d, 1H, aromatic), 7.13-7.20 (m, 2H aromatic), 7.14-7.46(m, 2H,aromatic), 7.69 7.7(d, 1H, aromatic), 8.61(s,1 H,amide)^{13}CNMR(100MHz,$CDCl_3$):32.4,84.8,109.3,117.8 ,119.8,121.4,123.0,126.2,128.7,128.9,133.5,144.0,147. 2,159.4,164.8,168.2,168.8
MS:m/z:400.05(100.0%),401.05(19.6%),402.04(4.5%),4 02.05(3.3%),401.04(1.5%)
. Elemental analysis: $C_{17}H_{12}N_4O_6S$ Calculated Values: C-51.00, H-3.02, N-13.99, S-8.01: ObservedValues:C-50.98, H-3.00, N-13.96, S-8.00

VI(k) 2- hydroxy-N-(7-Nitro-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl) benzamide

IR spectrum(KBr, cm^{-1}): 3058.12(C-H aromatic), 1682.02(C=Ostr), 1645.39(C=Nstr), 1532.06(C=C aromatic), 668.32 (S-CHstr); ^{1}HNMR (400MHz,CDCl3,δ,ppm): 3.34(s, 2H,CH_2), 6.54-6.55(s, 2H, aromatic OH), 7.04-7.05(d,1H,aromatic), 7.20-7.22(m,2H aromatic), 7.34-7.36 (m,2H,aromatic), 7.69-7.7(d, 1H, aromatic), 8.61(s, 1H, amide) ^{13}CNMR(100MHz,CDCl3): 32.4, 84.8, 117.8, 119.8, 121.4, 124.2,125.7, 128.7, 128.9, 130.6, 133.5, 135.9, 138.9, 159.4, 164.8, 168.2, 168.8. MS:m/z:400.05(100.0%),401.05(19.6%), Elemental analysis: $C_{17}H_{12}N_4O_6S$ Calculated Values: C-51.0, H-3.02, N-13.99, S-8.01: Observed Values: C-50.98, H-3.01, N-13.97, S-8.00.

VI(l) 2- hydroxy-N-(5-Hydroxy-2,4^1-dioxospiro[indoline-3,2^1-thiazolidin]-3^1-yl) benzamide

IR spectrum(KBr, cm^{-1}) :3069.12(C-H aromatic), 1682.02(C=Ostr), 1620.39(C=Nstr), 1520.06(C=C aromatic), 680.32 (S-CHstr); ^{1}HNMR (400MHz, CDCl3,δ, ppm): 3.44(s, 2H, CH_2), 6.97-6.98 (s, 2H, aromatic OH), 7.04-7.05(d, 1H, aromatic), 7.13-7.20 (m, 2H aromatic),7.14-7.46(m,2H,aromatic), 7.69-7.7 (d, 1H, aromatic), 8.61(s, 1H, amide) ^{13}CNMR(100MHz,CDCl3): 32.4, 86.1, 111.9, 115.0, 115.5, 117.8, 119.8, 121.4, 128.9, 129.2, 133.5, 133.7, 153.1, 159.4, 164.8, 168.2, 168.8. MS:m/z:371.06 (100.0%), 372.06 (19.5%), 373.05(4.5%), Elemental analysis: $C_{17}H_{13}N_3O_5S$ Calculated Values: C-54.98, H-3.53, N-11.31, S-8.63: Observed Values: C54.96-, H-3.51, N-11.28, S-8.60.

4. BIOLOGICAL ACTIVITIES:

In Vitro Anti-Inflammatory Activity by using TMPD Assay method for this Cayman's Colorimetric COX (Ovine) Inhibitor screening Assay Kit. The results were depicted in Table1.

Evaluation of anti-inflammatory activity

***In vitro* Anti-inflammatory activity**

The synthesized compounds were evaluated for their *in vitro* anti-inflammatory activity by TMPD assay method. [4]. This assay is based on chromogenic assay based on oxidation of N, N, N', N, -tetra methyl-p-phenylenediamine (TMPD) during the reduction of prostaglandinH2 by COX-2 enzyme. This measures the peroxides component of cyclooxygenases. The peroxide activity is assayed calorimetrically by monitoring the appearance of oxidized N, N, N', N, -tetramathyl-p-phenylenediamine (TMPD) at 590nm. The final volume of the assay was 220µl. All the wells background wells contain 160µl of assay buffer and 10µl of heme and 10µl of enzyme. The inhibitor wells contain150µl of assay buffer and 10µl of heme, 10µl of enzyme and 10µl of inhibitor. The plate was shaken for a few seconds and incubated for five minutes at 25^0C.Then 20µl of colorimetric substance, 20µl of arachidonic acid were added. The plate was again shaken for a few seconds and incubated for five minutes at 25^0C.Then the absorbance was noted at 590nm using plate reader.

Table:1: *In vitro* anti-inflammatory activity:

S.No	Compound	R	COX-2 Inhibition
1	VIa	H	46.23±0.38
2	VIb	5-Cl	65.33±0.14
3	VIc	7-Cl	61.19±0.24
4	VId	5-CH_3	49.23±0.14
5	VIe	7-CH_3	48.24±0.15
6	VIf	5-F	67.21±0.23
7	VIg	7-F	57.16±0.35
8	VIh	5-Br	62.23±0.22
9	VIi	5,6-Dichloro	70.24±0.35
10	VIj	5-NO_2	55.17±0.33
11	VIk	7-NO_2	52.15±0.22
12	VIl	5-OH	59.18±0.11
13		Indomethacin	73.32±0.32

***In Vivo* Anti-Inflammatory Activity**

The compounds which are showing best activity by in-vitro anti-inflammatory activity 6 Compounds were selected and were evaluated for in vivo anti-inflammatory activity at a dose range of 100mg/kg body weight by carragenan induced rat paw edema method, From the data it was reveals that all the tested compounds significantly reduced carrageenan induced edema and the results were presented in Table.2 and 3.

Table 2: *In vivo* anti-inflammatory activity

S.No	Compound	R	Mean Paw Edema Volume in ml ± SD			
			1h	2h	3h	4h
1	VIb	5-Cl	0.43	0.37	0.33	0.28
2	VIc	7-Cl	0.47	0.43	0.40	0.37
3	VIf	5-F	0.42	0.39	0.35	0.26
4	VIh	5-Br	0.45	0.39	0.34	0.29
5	VIi	5,6-Di chloro	0.34	0.29	0.26	0.23
6	VIl	5-OH	0.48	0.44	0.41	0.38
7	**Control Group**		0.56	0.63	0.71	0.80
8	**Indomethacin**		**0.35± 0.070**	**0.33 ± 0.120**	**0.28 ± 0.080**	**0.21 ± 0.062**

Table 3: *In vivo* anti-inflammatory activity

S.No	Compound	R	% Inhibition of Paw Edema			
			1h	2h	3h	4h
1	VIb	5-Cl	23.31	41.27	53.52	65
2	VIc	7-Cl	16.07	31.75	43.66	53.75
3	VIf	5-F	25.0	38.1	50.7	67.5
4	VIh	5-Br	19.63	38.1	52.11	63.75
5	VIi	5,6-Di chloro	39.29	53.97	63.38	71.25
6	VII	5-OH	14.29	30.16	42.25	52.5
7	**Indomethacin**		37.5	47.6	60.5	73.7

5. RESULTS AND DISCUSSION:

Some of the new isatin derivatives were obtained by cyclization of 2-hydroxy –N'[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene] benzohydrazide with thioglycollic acid in presence of glacial acetic acid depicted in scheme 1. Physical data of all the synthesized compounds are shown in Table1.

5.1. IN-VITRO ANTIINFLAMMATORY ACTIVITY:

The newly synthesized compounds were screened for in *vitro* anti-inflammatory activity. The assay was performed using colorimetric COX (ovine) inhibitor screening assay kit (Cyaman chemical, MI, USA). The colorimetric COX (ovine) inhibitor screening assay utilizes the peroxidase activity of ovine cyclooxygenase to oxidize the colorimetric substrate, N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD). The results were presented in Table 1. Among the tested compounds VIi(R=5,6-dichloro), VIf(R=5-F), VIb(R=5-Cl),VIh(R=5-Br),andVIc(R=7-Cl) are considered to possess more potent anti-inflammatory activity when compared to standard drug indomethacin.

5.2.IN-VIVO ANTIINFLAMMATORY ACTIVITY:

From the compounds which have shown best in-vitro anti-inflammatory activity, 6 Compounds were selected and were evaluated for in vivo anti-inflammatory activity at a dose range of 100mg/kg body weight by carrageenan induced rat paw edema method, From the data it was revealed that all the tested compounds significantly reduced carrageenan induced edema and the results were presented in Table2 and 3. Among the tested compounds VIi(R=5,6-dichloro),VIf(R=5-F),VIb(R=5=Cl) and VIH(R=5-Br) are considered to possess potent anti-inflammatory activity when compared to standard drug indomethacin. From the obtained results it is clear that di halo substituted derivative (VIi(R=5,6-dichloro)) is found to be more potent compared to other synthesized compounds.

CONCLUSION:

The present study involves synthesis and evaluation of 2- hydroxy-N- (2, 4^1-dioxospiro [indoline-3, 2^1-thiazolidin]-3^1-yl) benzamides for *In vitro*, *In-vivo* anti-inflammatory activities. The title compounds have shown potent anti-inflammatory activities.

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