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THE *IN-VITRO* ANTI-DENATURATION EFFECTS INDUCED BY SUBSTITUTED OXY/THIOXY PYRIMIDINE DERIVATIVES IN BOVINE SERUM ALBUMIN IS PROPOSED AS A SCREENING ASSAY FOR THE DETECTION OF ANTI-INFLAMMATORY COMPOUNDS WITHOUT THE USE OF ANIMALS

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ABSTRACT: A series of novel 2-{[6-(2-substituted Phenyl)-2-oxo-1, 2-dihydropyrimidin -4-yl]oxy} benzoic acid (2a-b)/ 2-{[6-(2-substituted Phenyl)-2 thioxo-1, 2-dihydropyrimidin-4-yl]oxy} benzoic acid (2c-d) were synthesized and tested for their *in-vitro* protein denaturation activity. Compound 2d was found to be promising and was more potent than the Acetylsalicylic acid (NSAID) in the inhibition of Bovine serum albumin denaturation. The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (1H NMR, IR, and MS) of all the synthesized compounds were in full agreement with the proposed structures.

INTRODUCTION: Pyrimidine are versatile Nitrogen-containing heterocyclic compounds possessing broad-spectrum of biological activity and oxopyrimidine, and Thioxopyrimidine has proven record of biological activities, which contains two Nitrogen atoms [1, 2]. They are known to exhibit pharmacological activities such as analgesic, anti-inflammatory, anti-arrhythmic, anti-parkinsonian, and anticancer activities [3-7]. Acetylsalicylic acid (Aspirin) is a very useful unit in the fields of medicinal and pharmaceutical chemistry and has been reported to exhibit a variety of biological activities. Chalcones and their analogies having α, β- Unsaturated carbonyl system are very versatile substrates for the evolution of various reactions and physiologically active compounds chalcones display a large number of different biological activities, such as antibacterial, antifungal, anticancer, anti-inflammatory, analgesic, antiviral, antimalarial, antipyretic and cytotoxic activities [8, 9].

The reaction of the urea/thiourea with the different chalcones of acetylsalicylic acid derivative resulting oxo / thioxy pyrimidine ring. In view of the pharmacological profiles of these two chemical moieties as described above, we considered it interesting to synthesized two chemically different but pharmacologically compatible molecules with the aim of obtaining some novel heterocyclic systems with potentially enhanced biological properties. In the present investigation a new series of novel 2-{[6-(2-substituted Phenyl)-2oxo-1, 2-dihydropyrimidin-4-yl]oxy} benzoic acid (2a-b)/ 2-{[6- (2-substituted Phenyl)-2-thioxo-1, 2-dihydro-pyrimidin-4-yl]oxy} benzoic acid (2c-d) were synthesized and evaluated for their *in-vitro* protein denaturation activity [10].

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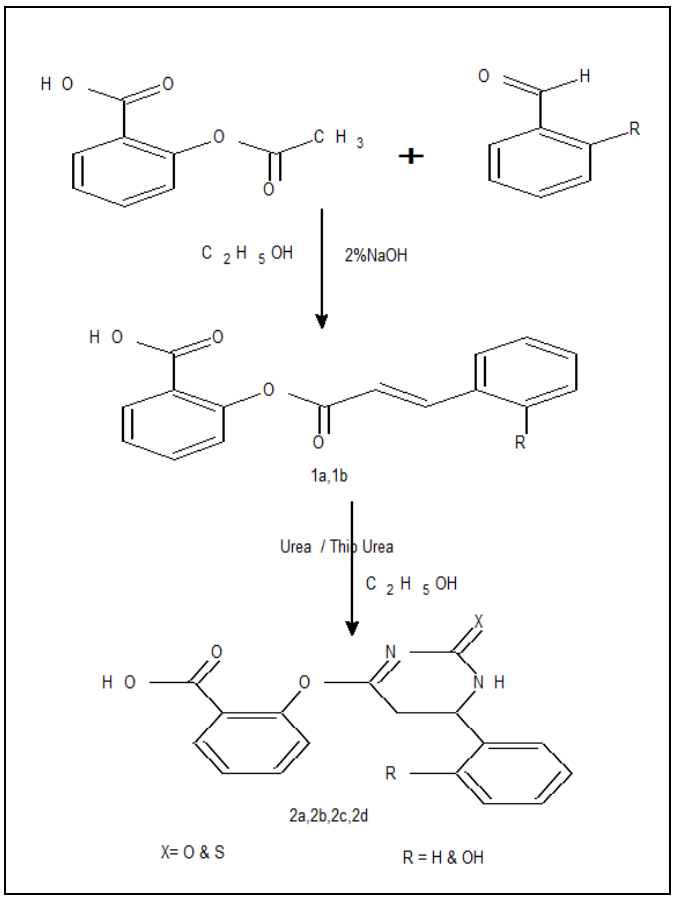

SCHEME 1

MATERIALS AND METHODS: Melting points were determined on a Tempstar apparatus and are uncorrected. Infrared spectra were recorded on a Jasco (410) FT-infrared spectrophotometer, measured as KBr disks. 1H NMR were recorded on a Bruker DPX-300 MHz spectrometer in deuteriochloroform with trimethylsilane as internal standard (chemical shift in ppm). The mass spectral data were obtained with a Perkin-Elmer Hitachi RMU-6L MS-30 spectrometer at 70 eV and a 90 °C inlet temperature. Purity of all the compounds was checked on silica gel plates and spots were located in iodine vapours. Elemental analysis was performed on EURO EA (Italy) analyzer and the results were within 0.3% of calculated values. Materials that were used in this study consists of acetylsalicylic acid ($C_9H_8O_4$), ethanol (C_2H_6O), benzaldehyde ($C_7H_60_2$), sodium hydroxide (NaOH), bovine serum albumin, hydrochloric acid (HCl) and urea (CON_2H_4) (Merck).

General Method for the Preparation of 2-{-3-phenylprop-2-enoyloxy} benzoic acid (1a): Equimolar quantity of acetylsalicylic acid (0.01 m) and benzaldehyde (0.01 m) were added in ethanol. 10 ml of 2% sodium hydroxide solution were added dropwise with constant stirring in a magnetic stirrer over a period of 30 min. The reaction mixture was stirred for 17 h and then refluxed for 6 h. then the reaction mixture was poured into ice cold water. The separated solid was filtered, washed with aqueous ethanol and recrystallized from ethanol: water (50:50). Compound 1b was prepared similarly.

2-{-3-phenylprop-2-enoyloxy} benzoic acid (1a): IR (KBr): 3076 (Ar-CH), 1732 (C=O), 1670 (C=O), 1609 (C=C), MS: (m/z) 252(M+); 1H NMR ($CDCl_3$) δ: 11.69 (s, 1H, COOH), 7.61(d, CH, CH=CH), 7.68 (d, CH, CH=CH), 8.06 (m, 9H, ArH), 6.02-7.58 (m, 4H, ArH);

2-[3-(2-hydroxyphenyl)prop-2-enoyl]benzoic acid (1b): IR (KBr): 3470 (OH), 3072(Ar-CH), 1730 (C=O), 1672 (C=O), 1602 (C=C), MS: (m/z) 268(M+); 1H NMR ($CDCl_3$) δ: 11.68 (s, 1H, COOH), 7.66 (d, 1H, CH=CH), 7.74 (d, 1H, CH=CH), 8.06 (m, 9H, ArH), 6.02-7.58 (m, 4H, ArH), 5.69 (s, IH, OH).

General Method for the Preparation of 2-{[6-2-hydroxyphenyl) -2-oxo-1, 2-dihydropyrymidin-4-yl] oxy} benzoic acid (2a): Equimolors quantity of 1a (0.1 M) and urea (0.1 M) were mixed in ethanol. The reaction mixture was refluxed for 4 to 5 h. Then the reaction mixture was poured into ice cold water. The separated solid was filtered, washed with aqueous ethanol, and recrystallized from ethanol: water (50:50), Compound 2b was prepared similarly.

2-(2-oxo- 6- phenyl-1,2,5,6-tetrahydropyrimidin-4-yl)benzoic acid: (2a) IR (KBr): 3346 (NH), 3075 (Ar-CH), 1731 (C=O), 1682 (C=O), 1607 (C=N), MS: (m/z) 310(M+); 1H NMR ($CDCl_3$) δ: 11.72 (s, 1H, COOH), 7.52 (d, 1H, NH), 5.23 (q, 1H, CH), 3.24(d, 2H, CH_2), 6.02-7.49 (m, 4H, ArH).

2-[6-(2- hydroxyphenyl) -2-oxo-1, 2, 5, 6-tetrahydropyrimidin-4-yl] benzoic acid (2b): IR (KBr): 3471 (OH), 3344(NH), 3076(Ar-CH), 1732 (C=O), 1682 (C=O), 1610 (C=N), MS: (m/z) 326 (M+); 1H NMR ($CDCl_3$) δ: 11.71 (s, 1H, COOH), 7.51 (d, 1H, NH), 5.19(q,1H,CH), 3.22 (d, 2H, CH_2), 6.02-7.47 (m, 4H, ArH), 5.66 (s, IH, OH).

General method for the preparation of 2-(6-phenyl-2-thioxo-1, 2, 5, 6-tetrahydropyrimidin-4-yl) benzoic acid (2c): Equimolors quantity of 1a (0.1 M) and urea (0.1 M) were mixed in ethanol. The reaction mixture was refluxed for 4 to 5 h. Then the reaction mixture was poured into ice cold water. The separated solid was filtered, washed with aqueous ethanol and recrystallized from ethanol: water (50:50), Compound 2d was prepared similarly.

2-{[6-2- hydroxyphenyl) -2-thioxo-1, 2-dihydropyrymidin-4-yl] oxy} benzoic acid (2c): IR (KBr): 3345(NH), 3075 (Ar-CH), 1730 (C=O), 1682 (C=O), 1609 (C=N), MS: (m/z) 326(M+); 1H NMR ($CDCl_3$) δ: 11.74 (s, 1 H, COOH), 7.57 (d, 1H, NH), 5.29 (q, 1H, CH), 3.28 (d, 2H, CH_2), 6.08-7.47 (m, 4H, ArH).

2-[6-(2- hydroxyphenyl)-2-thioxo-1, 2, 5, 6-tetrahydropyrimidin-4-yl]benzoic acid (2d): IR (KBr): 3473 (OH), 3342(NH), 3076(Ar-CH), 1733 (C=O), 1684 (C=O), 1612 (C=N), MS: (m/z) 342 (M+); 1H NMR ($CDCl_3$) δ: 11.72 (s, 1H, COOH), 7.56 (d, 1H, NH), 5.31(q,1H,CH), 3.32 (d, 2H, CH_2), 6.08-7.47 (m, 4H, ArH), 5.63 (s, IH, OH);

TABLE 1: PHYSICAL DATA OF SYNTHESIZED COMPOUNDS 1A-B & 2A-D

Compd.	R	Molecular Formula	M.P (C^0)	Yield (%)	% Analysis Calc. (Found)		
					C	H	N
1a	H	$C_{16}H_{12}O_4$	105	71	71.64 (71.62)	4.51 (4.56)	--------
1b	OH	$C_{16}H_{12}O_5$	99	67	67.60 (67.56)	4.25 (4.28)	--------
2a	H	$C_{17}H_{14}N_2O_4$	145	69	65.80 (65.82)	4.50 (4.51)	9.03 (9.07)
2b	OH	$C_{17}H_{14}N_2O_5$	141	71	62.57 (62.58)	4.32 (4.28)	8.59 (8.55)
2c	H	$C_{17}H_{14}N_2O_3S$	146	70	62.56 (62.57)	4.32 (4.33)	8.58 (8.52)
2d	OH	$C_{17}H_{14}N_2O_4S$	143	71	59.64 (59.61)	4.12 (4.10)	8.18 (8.21)

Inhibition of Protein Denaturation: The reaction mixtures (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of test compound (100 and 250 mg/ml of final volume). pH was adjusted at 6.3 using a small amount of IN HCl. The samples were incubated at 37 ºC for 3 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. For control tests, 0.05ml distilled water was used instead of synthesized product control tests that lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows

100 - (O.D. of test – O.D. of product control) / O.D. of control × 100

TABLE 2: *IN-VITRO* ANTI-INFLAMMATORY SCREENING OF SYNTHESIZED COMPOUNDS BY BOVINE SERUM ALBUMIN DENATURATION

Compound	Absorbance value (660 nm)*	Protein denaturation Mean (%)
2a	0.194	25
2b	0.185	34
2c	0.173	44
2d	0.155	60
Aspirin	0.149	66
Control	0.111	00

*Average of Three readings

RESULTS AND DISCUSSION: 2-{-3-phenylprop-2-enoyloxy} benzoic acid (1a) and 2-[3-(2-hydroxyphenyl) prop-2-enoyl] benzoic acid 1b were prepared by reacting with aspirin and substituted benzaldehyde. Compounds 1a,b were condensed with urea or thiourea in ethanol to afford the corresponding pyrimidine derivatives 2a, 2b or 2c, 2d in 69-71% yields **Table 1**. All the synthesized compounds were characterized by their elemental analysis, FT-IR, 1H NMR, and mass spectroscopy. Various 2-{[6-(2- substituted Phenyl) -2oxo-1, 2-dihydropyrimidin-4-yl] oxy} benzoic acid (2a-b)/ 2-{[6-(2-substituted Phenyl)-2oxo-1, 2-dihydropyrimidin-4-yl]oxy} benzoic acid (2c-d) were tested for their inhibition of protein denaturation activity by BSA assay method [8] using Bovine Serum Albumin.

Among the 4 compounds synthesized, two compounds exhibited significant activity. Hence, we can conclude that the size of hydroxyl substituents at the position of thioxy pyrimidine moiety is important for *in-vitro* anti-inflammatory activity. Among the newer derivatives, compound 2d showed promising activity in the test. It is conceivable that thioxy derivatives showing *in-vitro* Protein denaturation activity can be further modified to achieve NSAID agents with antiarthritis activity.

CONCLUSION: All the compounds have been screened for their *in-vitro* anti-inflammatory activity, advantage of present proposed research work was cost effective as compared to the *in-vivo* method.

Generally, it can be concluded that 2-[6-(2-hydroxyphenyl)- 2- thioxo-1, 2, 5, 6-tetrahydro-pyrimidin-4-yl] benzoic acid (2d) represent a new distinct classes of anti-inflammatory agents with antiarthritis activity. Compound 2d was found to be promising compared to the Acetylsalicylic acid (NSAID) in the inhibition of Bovine serum albumin denaturation. Further investigations with appropriate structural modifications of title compounds may result in therapeutically useful products.

All this is supremely encouraging for further research in this arena of anti-inflammatory drug discovery.

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CONFLICTS OF INTEREST: Nil

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