Design, synthesis, and biological evaluation of novel pyrido[2,3-*d*]pyrimidines containing derivatives

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Abstract

We have synthesized some new pyrido[2,3-d]pyrimidines containing some imines and 4-thiazolidinone derivatives by applying standard Vilsmeier-Haack reaction condition, reaction of N-(2,6-dimethoxypyrimidin-4-yl)acetamide (2) with Vilsmeier-Haack reagents gives 7-chloro-2,4 - dimethoxy pyrido[2,3-d]pyrimidine-6-carbaldehyde (3) which on further react with morpholine (4) and various substituted heteroamines (6a-d) gives 2,4-dimethoxy-7-morpholinopyrido[2,3-d] pyrimidine-6-carbaldehyde (5) and substituted Schiff bases (7a-d) respectively. Finally the reaction of various Schiff bases (7a-d) with thioglycolic acid and thiolactic acid gives 2,3-disubstituted-4-thiazolidinone (8a-d) and 2,3-disubstituted-5-methyl-4-thiazolidinone (9a-d) derivatives respectively. The best reaction condition for this goal was achieved by using acidic reaction condition. The salient features of this reaction are (1) it tolerates a wide range of functional groups, (2) easy to handle and required mild reaction conditions. Synthesized and characterised by elemental analyses and spectroscopic techniques. Stirred from literature survey of COX-1 and COX-2 enzyme inhibition study we establish that benzothiazole type structure is more suitable to inhibit concentration of COX-1 and COX-2 enzymes and to testing in vitro COX-1 and COX-2 inhibition study for synthesised derivatives. This study identified the potential compounds, which can be capable leads for their subsequent development as newer anti-inflammatory agents.

Keywords: *pyrido[2,3-d]pyrimidines, COX-1/COX-2 inhibition activity, Vilsmeier-Haack reaction.*

I. INTRODUCTION

A convenient and expeditious synthesis of biologically active molecules is one of the main challenges in medicinal chemistry[1]. The importance of heterocyclic compounds has long been recognized in the field of medicinal chemistry. Heterocyclic compounds are an integral part of the chemical and life sciences and constitute a considerable quantum of the modern research that is being currently pursued throughout the world[2]. Research on new substances possessing antibacterial activity has considerable attention owing to the continuous increase in bacterial resistance[3]. Here Nonsteroidal anti- inflammatory drugs (NSAID's) works by changing the biosynthetic pathway of eicosanoids through inhibition of cyclooxygenase (COX) enzymes. COX catalyses transformation of polyunsaturated omega-6 fatty acid to prostanoids which hold prostaglandins, thromboxane and prostacyclin[4–6]. Prostanoids plays vital role in various gastrointestinal, cardiovascular, nervous and urogenital system. COX enzymes, COX-1 and COX-2 both enzymes produce prostaglandins that endorse inflammation, pain and fever. COX-1 is a cleaning enzyme which is accountable for the production of prostanoid in many tissues even as COX-2 being an inducible enzyme expressed at the site of inflammation, infection, and cancer, produces prostanoids which are responsible for disease pathogenesis[7–8]. However, lots of information reveals the unfavourable effect of classical NSAIDs that works through inhibition of COX-1 mostly includes gastrointestinal ulceration, bleeding, and some kidney problem[9]. On the other hand, Azomethine has key role in design and development of novel compounds having potent biological activities like anti-bacterial [10], anti-fungal[11], anti-tuberculosis[12], anti-HIV[13], anti-viral[14], anti-inflammatory[15]. Therefore, synthesis of Azomethine and converting into thiazolidin-4-one derivatives are also reported to have important biological activities such as anti-inflammatory[16], anti-tuberculosis[17], anti-cancer[18], anti-tumor[19], anti-HIV[20], anti-bactrerial[21], anti-fungal[22], anti-oxidant[23], anti-viral[24], anti convulsant[25], diuretics[26], nematicidal[27], anti-histaminic[28] activity etc.

Benzothiazole is a privileged heterocyclic scaffold which belongs to the family of bicyclic heterocyclic compounds having a benzene ring fused with a five-membered thiazole ring comprising nitrogen and sulphur atoms. In the last few years, some novel benzothiazoles have been developed with diverse biological activities and impotent therapeutic-function including anti-inflammatory[29], antidiabetic[30],antimalarial[31] and antimicrobial[32]. Looking to the medicinal magnitude of Schiff bases and 4-thiazolidinone , we

report here the synthesis of new class of heterocyclic molecules in which all of these moieties are present moreover try to develop potential bioactive molecules.

II. Results and discussion:

A. Chemistry

Typical the strategy acquired for the synthesis of 7a-d, 8a-d and 9a-d is depicted in Scheme 1. The attack of sulphur nucleophile on imine carbon followed by intramolecular cyclization with elimination of water gives 2,3-disubstituted-4-thiazolidinones (8a-d) and 2,3-disubstituted-5-methyl-4-thiazolidinones (9a-d) derivatives which were confirmed by spectral analysis.

The formation of titled compound was confirmed by their FT-IR, ^{1}H NMR, mass spectra as well as elemental analysis. As an example, in the IR spectrum of compound 7a, characteristic is the N=CH stretching vibration, which appear as an intense band at 1625 cm^{-1}. There was no absorption in between 3300-3400 cm^{-1} which confirmed that free amino group of pyridine ring is converted into a proposed imine. The structural element characteristic for the pyrimidine nucleus, namely; the stretching vibration band for the C=N stretching observed at 1563 cm^{-1}. Several bands appeared at 1472 and 3070 cm^{-1} are due to the stretching of C=C and C-H vibrations of aromatic ring moreover C-O-C linkage and C-N stretching observed at 1138 and 1375 in the presence of morpholine ring. Also group –OCH_3 stretching vibration band observed at 1188 cm^{-1}. The ^{1}H NMR spectrum of compound 7a did not only show the absence of NH_2 protons of pyridine ring as singlet signal at between δ 5-6 ppm but exerted a singlet at higher field at δ 6.96 ppm for -CH=N proton of the imine group. There was emphasized signal as triplet for the morpholine ring contain protons core at δ 3.3 ppm. The three singlet of methoxy group protons are observed at δ 3.9 ppm and remaining protons resonated in the region at δ 7.3-7.6 ppm as multiplet signal. The strong absorption band observed at between 1650-1750 and 600-700 cm^{-1} for the presence of cyclic amido C=O group and C-S-C linkage of thiazolidine unit in both 8a and 9d. There was no absorption in the region of 1605-1621 cm^{-1} which 54.5 signifying the disappearance of imine group in this structure. Moreover the compound 9d showed a strong absorption band at 1365 cm^{-1} due to the presence of the CH_3 group attached on the C-5 position of thiazolidine ring which also confirmed the cyclocondensation of imine. The ^{1}H NMR spectrum of compound 8a showed singlet at δ 4.44 due to protons of active methylene group of the thiazolidine ring. The ^{1}H NMR spectrum of compound 9d showed singlet peaks at δ 2.15 due to -CH_3 proton of the thiazolidine ring system. The disappearance of the N=CH proton at between δ 9-10 ppm and the appearance of a methine proton at between δ 7.42-8.10 ppm as singlet also supported presence of thiazolidine ring in compound 8a and 9d. The other remaining aromatic protons appeared as a multiplet signal at between δ 7.1-7.7 ppm along with singlet at between δ 3.4-3.9 ppm corresponding to the methoxy group protons. Further, mass spectra of all the title compounds showed molecular ion peak M^+ corresponding to their mass which is also in agreement with its proposed structure. The obtained elemental analysis values are in good agreement with theoretical data.

All the synthesised compounds were characterised by ^{1}HNMR, IR, MS and elemental analysis. After characterisation, we have tested synthesised molecules for COX-1 and COX-2 enzyme inhibition study and the result of the study is mentioned in following table-1.

B. Biology

a) *In vitro cyclooxygenase (COX) inhibition activity*

All the newly synthesized compounds were evaluated for their ability to inhibit COX-1 and COX-2 inhibition using colorimetric assay. The potency of the compounds was determined as IC_{50} (µM) is the concentration which causing 50% of enzyme inhibition. Moreover, the COX-2 selectivity index (SI values) was calculated using IC_{50} (COX-1) and IC_{50} (COX-2) and compared with the standard drug Celecoxib. The above discussed parameters are presented in Table-1. All the compounds exhibited good inhibitory activities against COX-2 (**IC_{50}** = 0.55 - 4.87µM) compared to COX-1 (**IC_{50}** =87.0 - 137.5µM) with excellent COX-2 selectivity indexes of 16.16-243.5. Four targeted compounds (**8c, 8d, 9c** and **9d**) were found as potent and selective COX-2 inhibitors. 4-methoxy Benzothiazole (**7a**, **IC_{50} =** 4.87 µM**)** was found to be inactive COX-2 inhibitor. Fascinatingly, compounds with 6-ethoxy and 6-nitro substituent (**7c, IC_{50} =** 1.89 µM, **7d, IC_{50}** =1.68µM) exhibited good potency for COX-2 compared to 6-methoxy derivative (**7b, IC_{50}** = 4.53µM). Compounds with 6-ethoxy and 6-nitro substituents (**8c, 8d**) showed excellent activity (**IC_{50}**=0.73, 0.67 µM) against COX-2. Compounds with 4-methoxy substituents (**8a, IC_{50}** = 1.82µM) and 6-methoxy substituents (**8b, IC_{50}** = 1.88µM) showed decrease in the potency than having 6-ethoxy and 6-nitro substituents. However, compounds with methoxy substituents (**9a, IC_{50}** =1.41, **9b,IC_{50}** = 1.96µM) exhibited modest potency. Compound 9c and 9d with 6-ethoxy and 6-nitro substituents positions exhibited excellent potency (**IC_{50}**=0.58, 0.55µM) compared to Celecoxib (**IC_{50}**=0.30µM).

III. Experimental Section

A. General comments

All starting materials were purchased from commercial suppliers and used without further purification. All synthesized compounds were characterized by 1H NMR, IR, MS as well as

elemental analysis. Melting point and boiling point were obtained in open capillaries on Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. 1H NMR spectra were recorded on a Bruker400 MHz model spectrometer using DMSO-d6 as a solvent and TMS as internal standard MS spectra were recorded on XEVO G2-XS QTOF spectrophotometer. The chemical shifts of 1H NMR was reported as parts per million (ppm) downfield from TMS (Me4Si). The splitting patterns are designated as follows; s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses (C, H, N) were performed using a Heraeus CarloErba 1180 CHN analyzer (Hanau, Germany).

Preparation of N-(2, 6-dimethoxypyrimidin-4-yl)acetamide (2): (0.01 mole) of compound (1) (10 mL) of Acetic anhydride and 1-2 drops of Acetic acid was added and the mixture was heated under reflux for 6 h. The reaction mixture was poured into crushed ice (200 g) with stirring. The separated solid was filtered off and washed thoroughly with water. The progress of reaction was monitored by TLC using ethyl acetate: hexane (6:4) as eluent. The solid product obtained was filtered, washed with water and dried. The crude product was purified by crystallization from acetone to get the title compound (2).

Preparation of 7-chloro-2, 4 –dimethoxy pyrido[2,3-*d*] pyrimidine-6-carbaldehyde (3): A mixture of compound (2) (0.810 mg, 5 M mol) in DMF (4.0 ml, 50 m mol), $POCl_3$ (0.5 ml, 5 m mol) was added at room temperature, producing a semi-solid mass. A clear solution appeared after stirring for 4 h at room temperature. It was further stirred for 6 h. The reaction mixture was poured into crushed ice (200 g) with stirring. The separated solid was filtered off and washed thoroughly with water. The progress of reaction was monitored by TLC using ethyl acetate: hexane (6:4) as eluent. The solid product obtained was filtered, washed with water and dried. The crude product was purified by crystallization from acetone to get the title compound (3).

Preparation of 2, 4 – dimethoxy-7-morpholinopyrido [2, 3-*d*] pyrimidine-6-carbaldehyde (5): A solution of morpholine (4) (1.75 g, 20 m mol) in 10 ml of dichloromethane was gradually added under stirring to an ice-cooled mixture of compound (3). After stirring for 30 min. at 0-5 °C the mixture was washed with 3x10 ml of water in order to remove unreacted morpholine and its salt. The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The dry, flake-like residue were recrystallized from 1, 4-dioxane. The progress of reaction was monitored by TLC using chloroform: methanol (9:1) as eluent. The crude product was purified by crystallization from acetone to get the title compound (5).

General Preparation of Substituted Schiff bases (7a-d): A solution of (4) (2.0 g, 20 m mol) in 20 ml ethanol was added to equimolecular quantities of an amine (6) add 1-2 drop of Acetic acid. The reaction mixture was refluxed for 3 h at 60-70 ^oC the separated solid was filtered off and washed thoroughly with water. The progress of reaction was monitored by TLC using ethyl acetate: hexane (6:4) as an eluent. The solid product obtained was filtered, washed with water and dried. The crude product was purified by crystallization from acetone to get the title compound (7).

General Preparation of 2,3-disubstituted-4-thiazolidinone (8a-d): A mixture of substituted Schiff bases (7) (3.56 g, 1 mol), DMF (50 ml), Pinch of $Zncl_2$ and thioglycolic acid (1.84 g, 2 mol) was refluxed for 6 - 8 hours. Excess solvent was distilled off under reduced pressure. Progress of the reaction was monitored by TLC using ethyl acetate: hexane (4:6). After the completion of the reaction it was cooled and the product was filtered, washed with dilute sodium bicarbonate solution to remove unreacted acid and dried over anhydrous Na_2SO_4 to get substituted 4-Thiazolidinones derivatives.

General Preparation of 2,3-disubstituted-5-methyl-4-thiazolidinone (9a-d): A mixture of substituted Schiff bases (7) (3.56 g, 1 mol), DMF (50 ml), Pinch of $Zncl_2$ and thiolactic acid (1.84g, 2 mol) was refluxed for 6-8 hours. Excess solvent was distilled off under reduced pressure. Progress of the reaction was monitored by TLC using ethyl acetate: hexane (4:6). After the completion of the reaction it was cooled and the product was filtered, washed with dilute sodium bicarbonate solution to remove unreacted acid and dried over anhydrous Na_2SO_4 to get substituted 4-Thiazolidinones derivatives.

Spectral data and physical data of all the synthesized compounds are given in Spectra analysis data.

***N*-(2, 6-dimethoxypyrimidin-4-yl) acetamide (2):** White solid, M.P: 107-110 ^oC; Yield: 82%; Anal. Calcd. For $C_8H_{11}N_3O_3$: C, 48.73; H, 5.62; N, 21.31%. Found C, 48.76; H, 5.65; N, 21.35%; IR (KBr, Vmax/cm^{-1}): 3076, 1593, 1591, 1370, 1681, 3179, 1177; ^1H NMR (400 MHz, DMSO, δ ppm): 9.52 (s, 1H, 2^0 amide), 2.54 (s, 3H, CH_3), 3.64 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 8.31 (s, 1H, Ar-H); MS m/z 198.08 (M^+ +1).

7-Chloro-2,4-dimethoxypyrido[2,3-*d*]pyrimidine-6-carbaldehyde (3): Light-yellow, M.P: 115-120^oC; Yield: 77%; Anal. Calcd. For $C_{10}H_8ClN_3O$: C, 47.35; H, 3.18; N, 16.57 %. Found C, 47.32; H, 3.15; N, 16.54%; IR KBr, Vmax/cm^{-1}: 3076, 1593, 1591, 1681, 2785 – 2845, 709, 1177; ^1H NMR (400 MHz, DMSO, δ ppm): 9.52 (s, 1H, -CHO), 3.61 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 8.33 (s, 1H, Ar-H); MS m/z 254.2 (M^+ +1).

2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidine-6-carbaldehyde (5): Light-yellow, M.P:102-105^oC; Yield: 70%; Anal. Calcd. For $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41%. Found C, 55.23; H, 5.33; N, 18.44%; IR KBr, Vmax/cm^{-1}: 3078, 1598, 1590,1681, 2785 –2845, 1136 , 1374; ^1H NMR

(400 MHz, DMSO, δ ppm): 9.52 (s, 1H, -CHO), 3.62 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 8.34 (s, 1H, Ar-H), 2.36 (t, 4H,CH_2 morpholine ring),2.42 (t, 4H, morpholine ring); MS m/z 305.11 (M^+ +1).

***(E)*-N-((2,4-dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)methylene)-4-methoxybenzo[d]thiazol-2-amine(7a):** Orange solid; M. P: 182-185 ^oC; Yield: 73%; Anal. Calcd. For $C_{22}H_{22}N_6O_4S$: C, 56.64; H, 4.75; N, 18.01%. Found C, 56.66; H, 4.73; N, 18.04%; IR KBr, Vmax/cm^{-1}: 3070, 1472, 1563, 1625, 1188, 1138, 1375; ^1H NMR (400 MHz, DMSO, δ ppm): 3.37 (t, 4H, CH_2 morpholine), 3.38 (t, 4H, CH_2 morpholine), 3.95 (s, 3H, -OCH_3), 3.97 (s, 3H, -OCH_3), 3.99 (s, 3H, -OCH_3), 7.28 (s, 1H, Ar-H), 6.96 (s, 1H, CH=N), 7.30-7.63 (m, 3H, Ar-H) ; MS m/z 467.1 (M^+ +1).

***(E)*-N-((2,4-dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)methylene)-6-methoxybenzo[d]thiazol-2-amine(7b):** Orange solid; M.P.: 187-190 ^oC; Yield: 74%; Anal. Calcd. For $C_{22}H_{22}N_6O_4S$: C, 56.64; H, 4.75; N, 18.01%. Found C, 56.61; H, 4.73; N, 18.04%; IR KBr, Vmax/cm^{-1}: 3078, 1475, 1557, 1628, 1184, 1135, 1372 ; ^1H NMR (400 MHz, DMSO, δ ppm): 3.34 (t, 4H, CH_2 morpholine), 3.36 (t, 4H, CH_2 morpholine), 3.94 (s, 3H, -OCH_3), 3.96 (s, 3H, -OCH_3), 3.98 (s, 3H, -OCH_3), 7.27 (s, 1H, Ar-H), 6.97 (s, 1H, CH=N), 7.32-7.66 (m,3H, Ar-H) ;MS m/z 467.3 (M^+ +1).

***(E)*-N-((2,4-dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)methylene)-6-ethoxybenzo[d]thiazol-2-amine(7c):**Orange solid; M.P.: 194-197 ^oC; Yield: 73%; Anal. Calcd. For $C_{23}H_{24}N_6O_4S$: C, 57.49; H, 5.03; N, 17.49%. Found C, 57.46; H, 5.06; N, 17.46%; IR KBr, Vmax/cm^{-1}: 3064, 1463,1553, 1618, 1198, 1122, 1378; ^1H NMR (400 MHz, DMSO, δ ppm): 3.34 (t, 4H, CH_2 morpholine), 3.35 (t, 4H, CH_2 morpholine), 3.96 (s, 3H, -OCH_3), 3.97 (s, 3H, -OCH_3), 7.26 (s, 1H, Ar-H),6.92 (s,1H, CH=N), 7.35-7.67 (m, 3H, Ar-H), 4.55 (q, 2H, CH_2), 1.60(t, 3H, CH_3) ; MS m/z 481.7 (M^+ +1).

***(E)*-N-((2,4-dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)methylene)-6-nitrobenzo[d]thiazol-2-amine (7d):** Orange solid; M.P.: 185-190 ^oC; Yield: 70%; Anal. Calcd. For $C_{21}H_{19}N_7O_5S$: C, 52.38; H, 3.98; N, 20.36%. Found C, 52.34; H, 3.96; N, 20.32%; IR KBr, Vmax/cm^{-1}: 3073, 1474, 1558, 1628, 1193,1136,1376,1350 ; ^1H NMR (400 MHz, DMSO, δ ppm): 3.39 (t, 4H, CH_2 morpholine), 3.40 (t, 4H, CH_2 morpholine), 3.89 (s, 3H, -OCH_3), 3.91 (s, 3H, -OCH_3), 7.21 (s,1H, Ar-H), 6.83 (s, 1H, CH=N), 7.16-7.92 (m, 3H, Ar-H) ; MS m/z 483.3 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(4-methoxybenzo[*d*]thiazol-2-yl)thiazolidin-4-one(8a): Radish solid; M.P: 167-170 ^oC; Yield: 71%; Anal. Calcd for $C_{24}H_{22}N_6O_4S_2$: C, 53.32; H, 4.47; N, 15.55%. Found C, 53.32; H,4.44; N, 15.51%; IR KBr, Vmax/cm^{-1}: 3060, 1466, 802, 1144, 1177, 1540, 1665, 685 ; ^1H NMR (400 MHz, DMSO, δ ppm): 3.39 (t, 4H, CH_2 morpholine), 3.41 (t, 4H, CH_2 morpholine), 3.94 (s, 3H, -OCH_3), 3.98 (s, 3H, -OCH_3), 3.99 (s, 3H, -OCH_3), 7.26 (s, 1H, Ar-H), 4.44 (s, 2H, CH_2), 5.23 (s, 1H, CH), 7.10-7.53 (m, 3H, Ar-H); MS m/z 541.4 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(6-methoxybenzo[d]thiazol-2-yl)thiazolidin-4-one (8b): Radish solid; M.P: 169-174 ^oC; Yield: 68%; Anal. Calcd for $C_{24}H_{22}N_6O_5S_2$: C, 53.32; H, 4.47; N, 15.55%. Found C, 53.37; H, 4.43; N, 15.52%; IR KBr, Vmax/cm^{-1}: 3065, 1465, 805, 1148, 1177, 1547, 1658, 682 ;^1H NMR (400 MHz, DMSO, δ ppm): 3.45 (t, 4H, CH_2, morpholine), 3.47 (t, 4H, CH_2, morpholine), 3.61 (s, 3H, -OCH_3), 3.65 (s, 3H, -OCH_3), 3.68 (s, 3H, -OCH_3), 7.23 (s, 1H, Ar-H), 4.48 (s, 2H, CH_2), 5.28 (s, 1H, CH), 7.10-7.62 (m, 3H, Ar-H); MS m/z 541.5 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(6-ethoxybenzo[d]thiazol-2-yl)thiazolidin-4-one (8c): Orange solid; M.P:164-168 ^oC; Yield: 75%; Anal. Calcd for $C_{25}H_{26}N_6O_5S_2$: C, 54.14; H, 4.72; N, 15.15%. Found C, 54.18; H,4.76; N, 15.18%; IR KBr, Vmax/cm^{-1}: 3064, 1467, 809, 1146, 1178, 1545, 1658, 681 ;^1H NMR (400 MHz, DMSO, δ ppm): 3.42 (t, 4H, CH_2, morpholine), 3.44 (t, 4H, CH_2, morpholine), 3.60 (s, 3H, -OCH_3), 3.63 (s, 3H, -OCH_3), 7.26 (s, 1H, Ar-H), 4.44 (s, 2H, CH_2), 5.23 (s, 1H, CH), 7.10-7.53 (m, 3H, Ar-H), 4.62 (q, 2H, CH_2), 1.72 (t, 3H, CH_3); MS m/z 555.2 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (8d): Lightyellow solid; M.P:155-160 ^oC; Yield: 68%; Anal. Calcd for $C_{23}H_{21}N_7O_6S_2$: C, 49.72; H, 3.81; N, 17.65%. Found C, 49.75; H,3.85; N, 17.62%; IR KBr, Vmax/cm^{-1}: 3062, 1469, 806, 1143, 1176, 1542, 1659, 679, 780; ^1H NMR (400 MHz, DMSO, δ ppm): 3.42 (t, 4H, CH_2, morpholine), 3.48 (t, 4H, CH_2, morpholine), 3.60 (s, 3H, -OCH_3), 3.64 (s, 3H, -OCH_3), 6.38 (s, 1H, Ar-H), 5.11 (s, 2H, CH_2), 8.42 (s, 1H, CH), 7.42-7.89 (m, 3H, Ar -H); MS m/z 556.1 (M^+ 1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(4-methoxybenzo[d]thiazol-2-yl)-5-methylthiazolidin-4-one (9a): Light-orange solid; M.P: 173-176 ^oC; Yield: 76%; Anal. Calcd for $C_{25}H_{26}N_6O_5S_2$: C, 54.14; H, 4.72; N, 15.15%. Found C, 54.16; H, 4.74; N,15.17%; IR KBr, Vmax/cm-1: 3079, 1566, 1679, 1356, 1548, 691, 1138, 1586, 1166, 1365; ^1H NMR (400 MHz, DMSO, δ ppm): 3.67 (t, 4H, CH_2 morpholine), 3.74 (t, 4H, CH_2 morpholine), 3.83 (s, 3H, -OCH_3), 3.93 (s, 3H, -OCH_3), 3.97 (s, 3H, -OCH_3), 2.32 (s, 3H, CH_3), 7.02 (s, 1H, Ar-H), 6.73 (s, 1H, CH), 6.45 (s, 1H, CH), 7.46-7.96 (m, 3H, Ar-H); MS m/z 555.2 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(6-methoxybenzo[d]thiazol-2-yl)-5-methylthiazolidin-4-one (9b): Light-orange solid; M.P: 160-164 ^0C; Yield: 79%; Anal. Calcd for $C_{25}H_{26}N_6O_5S_2$: C, 54.14; H, 4.72; N, 15.15%. Found C, 54.17; H, 4.75; N,15.18%; IR KBr, Vmax/cm^{-1}:

3016, 1530, 1687, 1366, 1557, 697, 1154, 1575, 1173, 1357; ^{1}H NMR (400 MHz, DMSO, δ ppm): 3.38 (t, 4H, CH_2, morpholine), 3.40 (t, 4H, CH_2, morpholine), 3.59 (s, 3H, -OCH_3), 3.62 (s, 3H, -OCH_3), 3.66 (s, 3H, -OCH_3), 2.18 (s, 3H, CH_3), 7.20 (s, 1H, Ar-H), 4.47 (s, 1H, CH), 5.23 (s, 1H, CH), 7.11-7.72 (m, 3H, Ar-H); MS m/z 555.3 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(6-ethoxybenzo[d]thiazol-2-yl)-5-methylthiazolidin-4-one (9c): Lightorange solid; M.P: 190-194 ^{0}C; Yield: 69%; Anal. Calcd for $C_{26}H_{28}N_6O_5S_2$: C, 54.16; H, 4.96; N, 14.78%. Found C, 54.93; H, 4.99; N, 14.75%; IR KBr, Vmax/cm^{-1}: 3050, 1554, 1694, 1360, 1566, 689, 1142, 1585, 1180, 1365; ^{1}H NMR (400 MHz, DMSO, δ ppm): 3.41 (t, 4H, CH_2, morpholine), 3.43 (t, 4H, CH_2, morpholine), 3.62 (s, 3H, -OCH_3), 3.66 (s, 3H, -OCH_3), 3.69 (s, 3H, CH_3), 2.17 (s, 3H,CH_3), 7.28 (s, 1H, Ar-H), 4.48 (s, 1H, CH), 5.23 (s, 1H, CH), 7.23-7.63 (m, 3H, Ar-H), 4.59 (q, 2H, CH_2); MS m/z 569.1 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-5-methyl-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (9d): Lightorange solid; M. P: 150-155 ^{0}C; Yield: 73%; Anal. Calcd for $C_{24}H_{23}N_7O_6S_2$: C, 50.61; H, 4.07; N, 17.21%. Found C, 50.64; H, 4.05; N,17.24%; IR KBr, Vmax/cm^{-1}: 3061, 1470, 808, 1147, 1178, 1547, 1665, 689 , 783; ^{1}H NMR (400 MHz, DMSO, δ ppm): 3.48 (t, 4H, CH_2, morpholine), 3.49 (t, 4H, CH_2, morpholine), 3.62 (s, 3H, -OCH_3), 3.64 (s, 3H, -OCH_3), 2.15 (s, 3H, CH_3), 6.35 (s, 1H, Ar-H), 5.20 (s, 1H, CH), 5.92 (s, 1H, CH), 7.32-7.67 (m, 3H, Ar -H); MS m/z 570.1 (M^+ +1).

B. In vitro COX-1 and COX-2 inhibition assay:

The *in vitro* ability of test compounds to inhibit COX-1 and COX-2 isoenzymes was carried out using Cayman colorimetric COX (ovine) inhibitor screening assay kit (catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions[35]. Briefly, the assay was performed using the reaction buffer solutions of 0.1 M TRIS-HCl buffer(pH 8.0) containing 1.0 mM hematin, 2.0 mM phenol as cofactors and 5 mM EDTA. The test samples were dissolved in DMSO and tested at concentrations of (0.01, 0.1, 1.0, 10.0, 50.0, and 100.0 mM in a final volume of 1 mL), reference compound (1 or 100 mM) or the vehicle (DMSO, 1.0%). A unit of ovine purified COX-1 or COX-2 was suspended in the reaction medium and preincubated for 5 minutes at room temperature. Then 5.0 mM arachidonic acid was added to start the reaction and incubated at 37^{o}C for 20 min. The COX reaction was stopped by the addition of 50 mL of 1 M HCl. Indomethacin and NS-398 were used as positive controls for the COX-1 and the COX-2 assay respectively[36]. The absorbance was measured by the ELISA reader "rainbow" (TECAN). Percentage (%) inhibition was calculated by comparing test compounds with the blank and calculated using following equation:

$$= ([PGE2]_{vehicle} - [PGE2]_{drug}) \times 100/[PGE2]_{vehicle}$$

The concentration of the test compound causing 50% inhibition (IC50, mM) was calculated from the concentration response curve using GraphPad PRISM. The samples were tested at six different concentrations in duplicate manner

TABLE I
***In vitro* cyclooxygenase (COX) inhibition**

Compound	*COX-1*[a] *IC_{50} (μM)*	*COX-2*[a] *IC_{50} (μM)*	*SI*[b]
7a	*87.0*	*4.87*	*16.16*
7b	*87.5*	*4.53*	*18.6*
7c	*88.3*	*1.89*	*44.77*
7d	*114.6*	*1.68*	*68.15*
8a	*130.3*	*1.82*	*64.42*
8b	*129.6*	*1.88*	*63.18*
8c	*124.6*	*0.73*	*188.9*
8d	*110.0*	*0.67*	*231.07*
9a	*126.3*	*1.41*	*82.21*
9b	*115.7*	*1.96*	*56.18*
9c	*137.5*	*0.58*	*226.8*
9d	*137.3*	*0.55*	*243.5*
Celecoxib	*>100*	*0.30*	*>303*

[a]IC-50 value is the compound concentration required to produce 50% inhibition of COX-1 or COX-2
[b]Selectivity index is the ratio of the IC50 of COX-1 and COX-2.

Figure-1: Numerous drugs containing Benzothiazole motif [33-34].

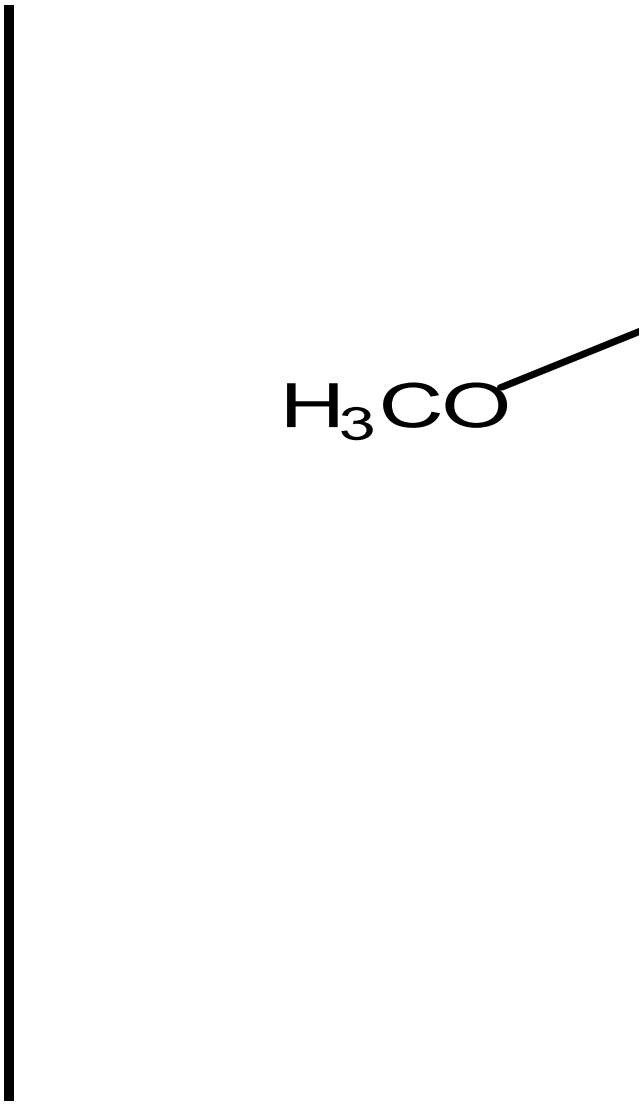

Figure-2: ***Synthetic procedure for the synthesis of new compound:***

CONCLUSIONS

In conclusion, we have verified a viable, extensive and very effective method for the synthesis of novel Schiff bases (7a-e), 2,3-disubstituted-4-thiazolidinone (8a-e) and 2,3-disubstituted-5-methyl-4-thiazolidinone (9a-e) derivatives was performed. The flexible reaction condition and straightforward development cause to be an enormously effective methodology. The scope of the reaction has been shown to endure both a diverse range of functionalized benzothiazole and a variety of heteroatom in fair to good yields. The results would be of immense interest because, of the insight used to improve the synthetic efficiency in modern organic chemistry, the combination of multi-step synthesis reaction has been standard as the especially effective way for atom-efficient and environmentally benign synthesis. Consequently, these methods are expected to find practical applications in pharmaceuticals, functional materials, and coordination chemistry. Further, the biological study showed that compound 8c, 8d, 9c and 9d exhibited remarkable COX-2 inhibition (IC_{50}:0.73, 0.67,0.58 and 0.55µM; SI:188.9, 213.07,226.8 and 243.5) comparable with standard drug Celecoxib (IC_{50}:0.30 µM ; SI: >303).

ACKNOWLEDGMENT

We thank to Applied Chemistry Department, SVNIT, Surat for providing research facilities. Authors are also thankful to RSIC Punjab University for the FTIR, ^{1}H NMR and MS as well as elemental analysis.

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