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SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-PHENYL THIOSEMICARBAZIDE DERIVATIVES AS ANTIINFLAMMATORY AGENT

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ABSTRACT

Thiosemicarbazides derivatives were synthesized by reacting 5-chloro-2-hydrazinylbenzo [d]oxazole and substituted phenyl isothiocyanate then characterized for Molecular formula, Physical state, Color, Melting point, Yield, Solubility and R_f – value. Structure of synthesized Thiosemicarbazides derivatives were elucidated using spectrogram data as well as physical method e.g. Elemental analysis, FT-IR, ^1H NMR and mass. Moreover, synthesized compounds characterized by the TLC and melting point. In addition, by visual inspection synthesized compounds also characterized. Novel synthesized Thiosemicarbazides derivatives were evaluated *in- vitro* for anti-inflammatory activity by kit available in market estimate the inhibition of Cox-1 and Cox-2 activity. Novel synthesized Thiosemicarbazides derivatives TSCZ-5 was exhibited excellent anti-inflammatory activity against COX-1 & Cox-2 with IC_{50} value less than <1mM. TSCZ-1, TSCZ-2 & TSCZ-4 possessed moderate activity against both COX-1 & Cox-2. TSCZ-3 exhibited poor activity against both COX-1 & Cox-2. But over all conclusion of anti-inflammatory study of Novel synthesized Thiosemicarbazides derivatives was that inhibition was increased with concentration of samples increased.

KEYWORDS: 4-phenyl Thiosemicarbazides, anti-inflammatory, Cox-1 and Cox-2.

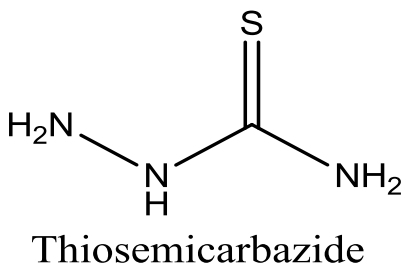
INTRODUCTION

Inflammation is a defensive mechanism in the body the immune system recognized damaged cells, irritant, and pathogens and protective response involving immune cell, blood vessel that serves as a mechanism initiating the elimination of noxious agent and of damage tissue inflammation is part of body's immune response. A Variety of chemical mediators from

circular system, inflammatory cells and injured tissue actively contributes to and adjust the inflammatory response the released chemical mediators include (1) Vasoactive amines such as histamine and serotonin (2) Peptide e.g. brdykinine (3) Eicosanoids e.g. thromboxanes, leukotrines and prostaglandins.[1] Inflammatory Disorder was included Autoimmune diseases, Acne vulgaris, chronic prostatitis, Glomerulonephritis, Inflammatory bowel disease, Rheumatoid arthritis, Transplant rejection.[2] They are many drugs available to decrease to joint pain, swelling, inflammation and possibly prevent, minimize the progression of the inflammatory disease, these include NSAIDs.[3] NSAIDs have been commanly used to reduce pain and inflammation in different arthritic and prospective condition. NSAIDs have four major activities e.g. anti-inflammatory, antipyretic, and analgesic. There are too many common and serious side effects of NSAIDs e.g. Gastritis/erosion if stomach lining,[4] Kidney damage,[5] Tinnitus,[6] Increased bleeding risk,[6] Allergic symptoms,[6] Fluid retention/ swelling,[7] cardiovascular disease/High blood pressure.[8]

Thiosemicarbazide (NH2-NH-CSNH2) is the simplest hydrazine derivative of thiocarbamic acid.[9] The chemical behaviour of thiosemicarbazide is alike to its correspondent semicarbazide, however, is of superior chemical adaptability of the thione group as compared with that of keto group and is liable for more diverse behavior of thiosemicarbazide.[10] Due to the existence of several reactive centres, these compounds are convenient precursors for the synthesis of nitrogen and sulfur containing heterocyclic compounds such as pyrazoles, thiazoles, thiadiazoles, thiadiazines, triazoles, pyrimidines, triazines, pyrazolotriazines, and thiazolotriazines and so on.[11] Due to the capability of thiosemicarbazide to form complexes with zinc, iron, nickel, copper, and other metalcat ions, which play an important role in biological processes.[12]

The imine bond (-N=CH-) in this compounds are useful in organic synthesis, in particular for the preparation of heterocycles and non-natural β-aminoacids.



Thiosemicarbazide

Figure 1: Chemical structure of thiosemicarbazide.

The major side effects of NSAIDs are their gastrointestinal ulcerogenic activity. COX-2 in contrast is induced in inflammatory cells in response. NSAIDs can cause ulcer in the stomach and promote bleeding. NSAID increase the risk potentially fatal, stomach and intestinal adverse reaction for example, bleeding, ulcers and perforation of stomach or intestines. Cause-2 inhibitors cause less bleeding and fewer ulcer than other NSAID drug Rofecoxib, Valdecoxib.

Therefore we are planning to synthesize novel anti-inflammatory derivatives having lesser side effect. Our aim is to synthesize 4-phenyl thiosemicarbazide derivative and evaluate them.

MATERIAL AND METHODS

To synthesize novel 4-phenyl thiosemicarbazide derivatives Isothiocyanatobenzene, 4-methyl Isothiocyanatobenzene, 4-methoxy Isothiocyanatobenzene, 4- chloro Isothiocyanatobenzene and 4-hydroxyIsothiocyanatobenzene were purchased from Sigma Aldrich Co. 5-chloro-2-hydrazinylbenzo[d]oxazole, NaOH and Hydrochloric Acid were obtained from Qualigens fine chemicals, Navi Mumbai. Ethanol, Chloroform, Methanol, Ether and Benzene purchased from Spectrochem Pvt. Ltd., Mumbai (India). Anti-inflammatory activity was evaluated by "COX-1 & 2 (human ovine) inhibitor Screening assay kit" obtained from Cayman, U.S.A.

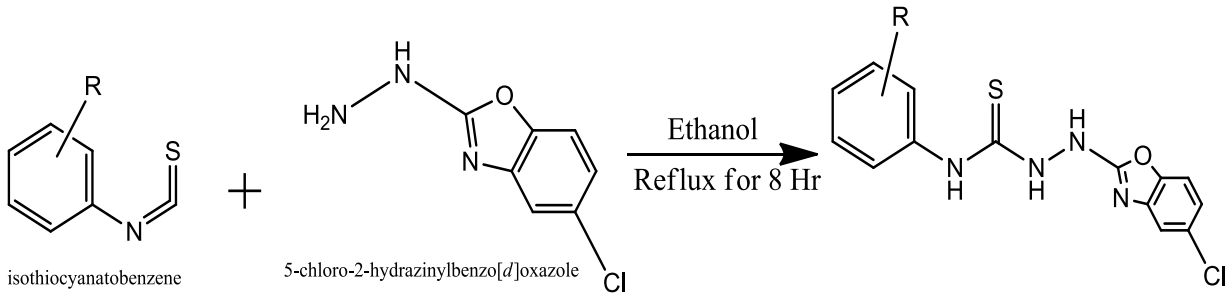
Fourier Transform Infrared Spectrophotometer (FTIR-RXI) by BURKER, Japan and ^{1}H-NMR Spectrometer JNM- ECX500 by JEOL Ltd. Japan.

Methods

General method of synthesis of thiosemicarbazides

5-chloro-2-hydrazinylbenzo[d]oxazole (0.02mol) was dissolved in 50 ml of ethanol (by heating) and substituted phenyl isothiocyanate (0.025mol) was heated under reflux on a water bath for 30min. Completion of reaction was determined by TLC. The reaction mixture was filtered still hot. After cooling to room temperature, precipitate appeared. The crude product was collected by filtration, washing with brain and recrystallized in ethanol to obtain the desired pure product.

Scheme of synthesis



Substitute (R) = -H, -CH_3, -OCH_3, -OH, -Cl

Figure 2: Scheme of synthesis of novel thiosemicarbazides.

Methods of characterization of newly synthesized compound

Physical evaluation of newly synthesized compound

Color, consistency, physical stat and smell were observed by visual inspection and recorded.

Calculation of % yield

Percentage of Yield was calculated by following formula:

$$\% \text{ Yield } = \frac{\text{Practical yield}}{\text{Theoretical yield}} \text{ X } 100$$

Determination of melting point

Melting points of newly synthesized compounds were determined by open capillary method using the digital melting point apparatus and were uncorrected. Compounds were placed in one end sealed capillary and placed in the caves made for the capillary. Thermometer was already placed in their caves because it is digital apparatus. The temperature at which compound start melting and the temperature at which it completely melts were recorded as the melting point range.

Solubility of compounds in different solvents

The various solvents such as water, ethanol, chloroform, ether, benzene, methanol, dimethyl formamide (DMF) and dimethyl sulphoxide (DMSO) were taken for dissolving intermediates and final products. 10 mg of each compound was weighed and added to 10 ml of each solvent individually taken in 50 ml beaker. The observation was recorded observed for different compounds.

Thin layer chromatography

Thin layer chromatographic analysis of compounds was done on silica gel G coated glass plates. The adsorbent silica gel G was coated to a thickness of about 0.3 mm on previously cleaned TLC plates of 20 x 10 cm. using conventional spreader. The plates were placed in hot air oven at 105 °C for 30 min. The solution of compounds was applied as a spot on the activated plate about 2 cm above from the lower edge. The mobile phases were selected according to the polarity of the products. $CHCl_3$:CH_3OH (9:1) is used as mobile phase. The spots were visualized by exposure to iodine vapor.

Methods of spectral analysis of newly synthesized compound

Elemental analysis

Elemental analysis of compounds was done on Vario micro elemental analyser (Elementar Germany).

FTIR spectral analysis

IR spectra of the compound were obtained from FT-IR spectrophotometer using KBr pellets, recorded on Bruker FT-IR.

Preparation of KBr pellet of compounds

100 mg of dehydrated KBr was accurately weighed. To this added 1 mg of compound and mixed well. The mixture was placed in an evacuable die and subjected to a pressure of 5-6 tons for 5 min. A transparent disc was produced which was then placed in a pellet holder and IR spectra were taken.

^{1}H-NMR spectral analysis

^{1}H NMR spectra of compounds was recorded on Bruker NMR spectrophotometer in deuterium- substituted chloroform as solvent of compounds and TMS (tetra methyl silane) was used as internal standard. Chemical shift was measured as delta-value (δ-value) in ppm (parts per million).

Method of *in-vitro* Anti-Inflammatory evaluation

COX Inhibition assay Kit

The assay was performed by using Colorimetric COX (human ovine) inhibitor Screening assay kit. Contains assay buffer, heme, enzyme COX-1and COX-2.

Preparation of test solutions

Accurately weighed amount of standard drug (aspirin) and novel synthesized semicarbazone derivatives was dissolved in some quantity of methanol and final volume was make up with water to produced 0.1mM, 1mM and 10mM.

COX Inhibition assay

Principle: The COX Inhibition assay utilized the peroxidase component of the COX catalytic domain. The peroxidase activity was assayed colorimetric method by monitoring the appearance of oxidized N, N, N, N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm.

General procedure: mixed well 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (either COX-1 or COX-2) then added 10 µl of different concentration (0.1 mM, 1 mM and 10 mM) of each synthesized derivatives as sample or standard drug. Aspirin (acetylsalicylic acid) was used as a standard drug.

Calculation: The percent COX inhibition was calculated using following equation:

$$\textbf{COX inhibition Activity}\ (\%) = 1 - \frac{T}{C} X\ 100$$

Where,

T = Absorbance of the inhibitor well at 590 nm.

C = Absorbance of the 100 % initial activity without inhibitor well at 590 nm.

RESULTS AND DISCUSSION

Characterization of novel synthesized thiosemicarbazides

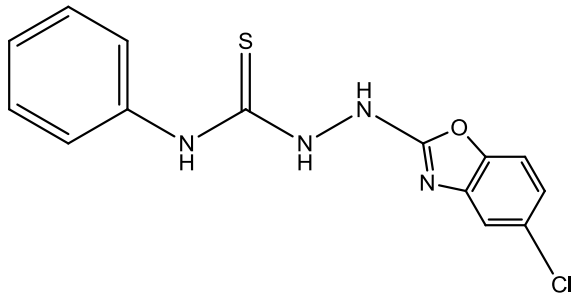
Table no. 1: General characteristics of novel synthesized thiosemicarbazides.

Properties	TSCZ-1	TSCZ-2	TSCZ-3	TSCZ-4	TSCZ-5
Molecular formula	$C_{14}H_{11}ClN_4OS$	$C_{15}H_{13}ClN_4OS$	$C_{15}H_{13}ClN_4O_2S$	$C_{14}H_{11}ClN_4O_2S$	$C_{14}H_{10}Cl_2N_4OS$
Molecular weight	318.78	332.05	348.81	334.78	353.32
Physical state	Prism shape Crystalline solid	Crystalline solid	Crystalline solid	Crystalline solid	Crystalline solid
Color	Yellowish	Reddish yellow	Yellowish	Dark Yellow	Off White
Melting point	171^{o}C	182 ^{o}C	192 ^{o}C	184-186^{o}C	174-176 ^{o}C
Yield	68.10%,	62.72%,	65.52 %,	69.32 %,	70.51%,
Solubility	Ethanol, DMSO and	Ethanol, DMSO and	Ethanol, DMSO and	Ethanol, DMSO and	Ethanol, DMSO and

	Methanol	Methanol	Methanol	Methanol	Methanol
R_f – Value	0.54	0.76	0.42	0.65	0.49

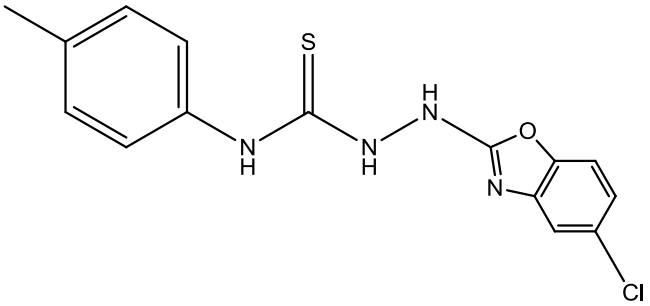
Structural Elucidation of Novel Synthesized Thiosemicarbazides

1. **TSCZ-1 (**2-(5-chlorobenzo[d]oxazol-2-yl)-N-phenylhydrazinecarbothioamide**)**



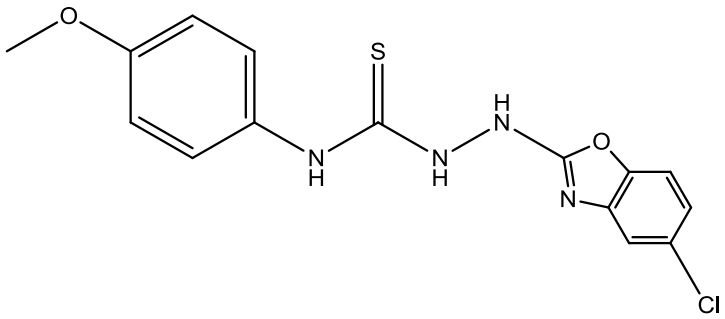
Elemental analysis calculated (Found) % for **$C_{14}H_{11}ClN_4OS$** : C, 52.75(52.64); H, 3.48(3.45); Cl, 11.12 (11.04); N, 17.58(17.41); O, 5.02 (5.01); S, 10.06(10.00) **FT-IR (KBr):cm^{-1}** 3051 **N-H** str., 1651 **C=N** str.(Ar), 1524 **C=C** str. (Ar), 1463 **C-H** str., 1308 **C-N** str., 1254 **C-O** str. **^{1}H NMR ($CDCl_3$, 500 MHz):** δ 7.81(s, 1H, CH), 7.74-7.71(m, 3H, CH), 7.48(d, 2H, *J* =2.65, CH-Ar), 7.23(t, 2H, *J* =8.05, 3.95, CH), 6.81(t, 1H, *J* =7.45, 5.00, CH), 4.01(s, 2H, NH), 2.02 (s, 1H,NH), ppm.**MS:** 318 (M+1)

2. **TSCZ-2 (**2-(5-chlorobenzo[d]oxazol-2-yl)-N-(p-tolyl) hydrazinecarbothioamide)



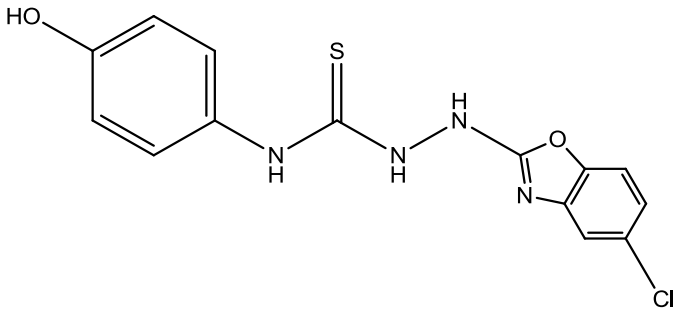
Elemental analysis calculated (Found) % for **$C_{15}H_{13}ClN_4OS$**: C, 54.13(54.01); H, 3.94(3.73); Cl, 10.65(10.42); N, 16.83(16.67); O, 4.81(4.74); S, 9.63(9.58). **FT-IR (KBr): cm^{-1}** 3052 **N-H** str., 1455 **C-H** str., 1436 **C-H** str.(methyl), 1651 **C=N** str., 1308 **C-N** str., 1254 **C-O** str. **^{1}H NMR ($CDCl_3$, 500 MHz):** δ 7.81(s, 1H, CH), 7.73(d, 1H, *J* =10.4, CH), 7.48(d, 1H, *J* = 7.15, CH), 6.96(d, 2H, *J* =10.8, CH), 6.39(d, 2H, *J* = 5.55, CH), 4.01(s, 2H, NH), 3.34(s, 3H, CH_3), 2.02 (s, 1H, NH), ppm. **MS:** 332 (M+1).

3. **TSCZ-3**(2-(5-chlorobenzo[d]oxazol-2-yl)-N-(4-methoxyphenyl)hydrazinecarbothioamide)



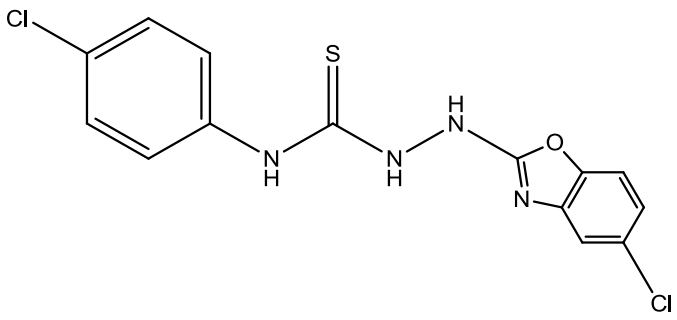
Elemental analysis calculated (Found) % for **$C_{15}H_{13}ClN_4O_2S$**: C, 51.65(51.56); H, 3.76(3.71); Cl, 10.16(10.07); N, 16.06(16.01); O, 9.17(9.11); S, 9.19(9.12). **FT-IR (KBr): cm^{-1}** 3072-**N-H** str., 1462-**C-H** str., 1400-**C-H** str.(methyl), 1682-**C=N** str., 1544-**C=C** str. (Ar), 1332-**C-N** str., 1266-**C-O** str. **^{1}H NMR ($CDCl_3$, 500 MHz):** δ 7.81(s, 1H, CH), 7.76(d, 1H, *J* = 8.8, CH), 7.50(d, 1H, *J* = 9.6, CH), 6.74(d, 2H, *J* =10.25, CH), 6.35(d, 2H, *J* = 8.05, CH), 4.01(s, 2H, NH), 3.83(s, 3H, O-CH_3), 2.00 (s, 1H, NH), ppm. **MS:** 348 (M+1).

4. TSCZ-4 (2-(5-chlorobenzo[d]oxazol-2-yl)-N-(4-hydroxyphenyl) hydrazinecarbothi oamide)



Elemental analysis calculated (Found) % for **$C_{14}H_{11}ClN_4O_2S$:** C, 50.23(50.17); H, 3.31(3.26); Cl, 10.59(10.51); N, 16.74(16.68); O, 9.56(9.50); S, 9.58(9.53). **FT-IR (KBr): cm^{-1}** 3513-**N-H** str., 3366- **O-H** str., 1556-**C=C** str.(Ar), 1405-**C-H** str. (methyl). **^{1}H NMR ($CDCl_3$, 500 MHz):** δ 7.80(s, 1H, CH), 7.75(d, 1H, *J* = 9.1, CH), 7.50(d, 1H, *J* = 11.1, CH), 6.70(d, 2H, *J* =11.05, CH), 6.30(d, 2H, *J* = 10.05, CH), 5.36(s, 1H, OH), 4.01(s, 2H, NH), 2.01 (s, 1H, NH), ppm. **MS:** 334 (M+1).

5. TSCZ-5 (2-(5-chlorobenzo[d]oxazol-2-yl)-N-(4-chlorophenyl)hydrazinecarbothioamide)



Elemental analysis calculated (Found) % for **$C_{14}H_{10}Cl_2N_4OS$:** C, 47.60(47.54); H, 2.85(2.79); Cl, 20.07(20.05); N, 15.86(15.80); O, 4.53(4.44); S, 9.08(9.02). **FT-IR (KBr): cm^{-1}** 3072-**N-H** str., 1462-**C-H** str. 1409-**C-H** str.(methyl), 1682-**C=N** str., 1544-**C=C** str.(Ar), 1332-**C-N** str., 1227-**C-O** str. **^{1}H NMR ($CDCl_3$, 500 MHz):** δ 7.81(s, 1H, CH), 7.74(d, 1H, *J* = 7.9, CH), 7.48(d, 1H, *J* = 6.95, CH), 7.24(d, 2H, *J* = 10.75, CH), 6.59(d, 2H, *J* = 10.55, CH), 5.36(s, 1H, OH), 4.00(s, 2H, NH), 2.00 (s, 1H, NH), ppm. **MS:** 353 (M+1).

***In-vitro* Anti-Inflammatory Evaluation by COX- Inhibition assay**

(A) COX- 1 Inhibition Assay

Table no. 2: Effect of novel synthesized thiosemicarbazides on COX-1.

S. No.	Code of Sample	% Inhibition in different Concentrations			IC $_{50}$ Value (mM)
		0.1mM	1mM	10mM	
1	TSCZ- 1	30.32 ± 0.74	48.64 ± 0.43	65.42 ± 0.55	> 1
2	TSCZ- 2	28.75 ± 0.33	36.73 ± 0.14	51.86 ± 1.75	> 1
3	TSCZ- 3	23.52 ± 1.32	35.46 ± 0.63	46.11 ± 0.75	> 10
4	TSCZ- 4	34.43 ± 0.92	42.53 ± 0.72	56.48 ± 1.43	> 1
5	TSCZ- 5	39.97 ± 0.23	**58.46 ± 0.69**	69.37 ± 0.12	**< 1**
6	Aspirin	47.72 ± 0.23	76.43 ± 0.43	93.52 ± 1.23	< 1

Results summarized are the mean values of n = 3 ± S.D

(B) COX- 2 Inhibition Assay

Table no. 3: Effect of novel synthesized thiosemicarbazides on COX-2.

S. No.	Code of Sample	% Inhibition in different Concentrations			IC $_{50}$ Value
		0.1mM	1mM	10mM	
1	TSCZ- 1	23.42 ± 1.30	49.23 ± 1.35	55.74 ± 0.37	> 1
2	TSCZ- 2	19.32 ± 0.12	25.63 ± 1.44	51.22 ± 0.74	< 10
3	TSCZ- 3	22.85 ± 0.43	32.32 ± 1.11	45.74 ± 1.85	> 10
4	TSCZ- 4	25.86 ± 0.84	38.12 ± 0.53	53.78 ± 0.65	< 10
5	TSCZ- 5	41.63 ± 0.64	51.23 ± 0.24	78.86 ± 1.43	< 1
6	Aspirin	48.43 ± 1.45	64.74 ± 0.13	83.32 ± 0.22	< 1

Results summarized are the mean values of n = 3 ± S.D

Statistical analysis

(A) Statistical Analysis of COX- 1 Inhibition Assay

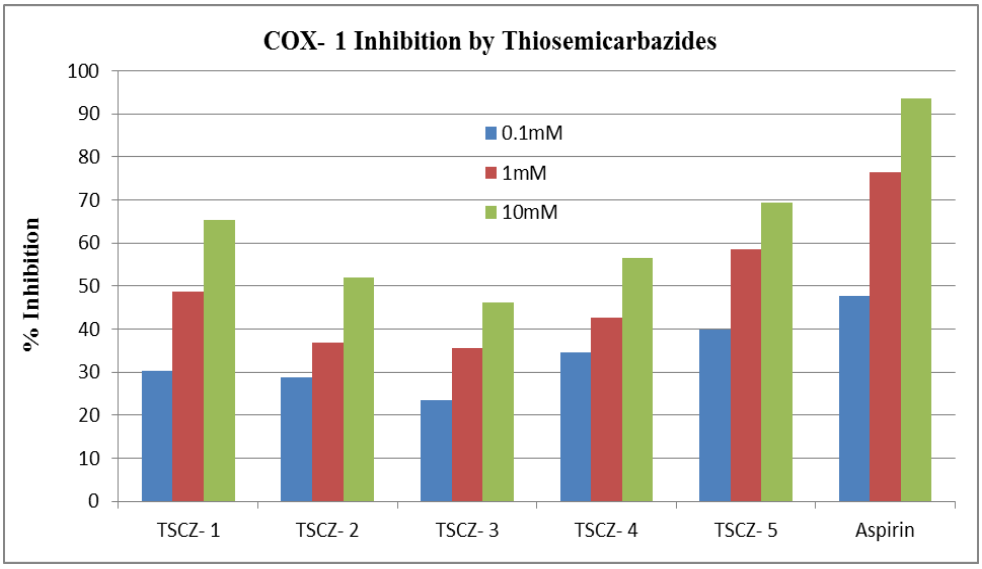


Figure 3: COX- 1 Inhibition assay.

(B) Statistical Analysis of COX- 1 Inhibition Assay

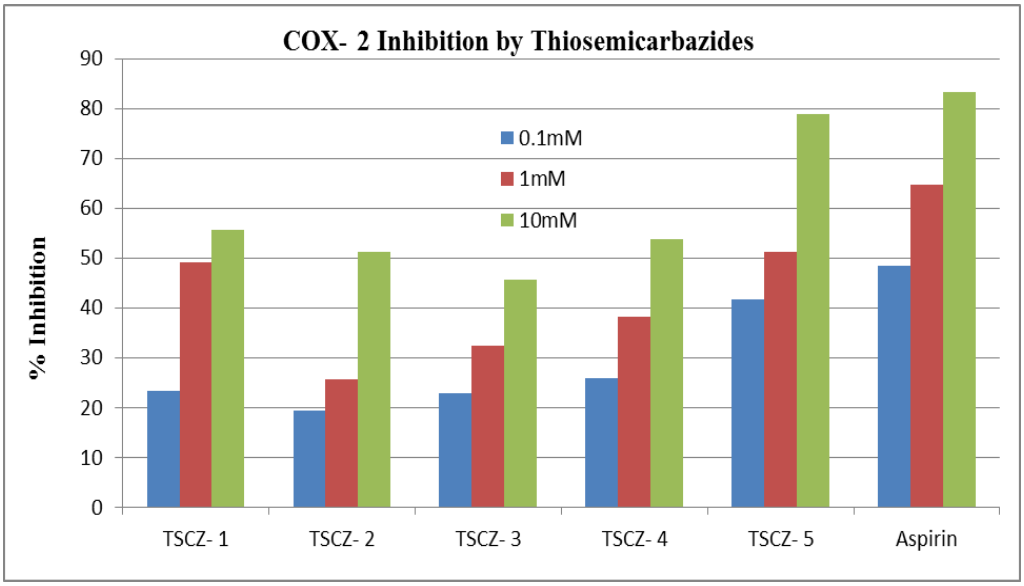


Figure 4: COX- 1 Inhibition assay.

DISCUSSION

Thiosemicarbazides derivatives were synthesized by reacting 5-chloro-2-hydrazinylbenzo [d]oxazole and substituted phenyl isothiocyanate then characterized for Molecular formula, Physical state, Color, Melting point, Yield, Solubility and R_f - value and structure of synthesized Thiosemicarbazides derivatives were elucidated using spectrogram data as well as physical method e.g Elemental analysis, FT-IR, ^{1}H NMR and mass. Appearance of

absorption bands in derivatives at 3260(-NH- N-H str.) which is present in all thiosemicarbazides in FT-IR spectrum clearly indicated. This fact was further supported by ^{1}H NMR spectrum, presence of peak at 4.00 & 2.00ppm of different derivative, corresponding to their structure. Moreover, synthesized compounds characterized by the TLC and melting point. In addition, by visual inspection synthesized compounds also characterized.

Novel synthesized Thiosemicarbazides derivatives were evaluated *in- vitro* for anti-inflammatory activity by kit available in market estimate the inhibition of Cox-1 and Cox-2 activity. Novel synthesized Thiosemicarbazides derivatives TSCZ-5 was exhibited excellent anti-inflammatory activity against COX-1 & Cox-2 with IC_{50} value less than <1mM. TSCZ-1, TSCZ-2 & TSCZ-4 possessed moderate activity against both COX-1 & Cox-2. TSCZ-3 exhibited poor activity against both COX-1 & Cox-2. But over all conclusion of anti-inflammatory study of Novel synthesized Thiosemicarbazides derivatives was that inhibition was increased with concentration of samples increased.

CONCLUSION

Thiosemicarbazides are interesting groups of heterocyclic compounds exhibiting diverse pharmacological activities. Reported structure based drug design too gives an emphasis on Thiosemicarbazides moiety. We thought that these models as such for synthesis give good opportunities to look for discovering ideal lead for anti-inflammatory activity.

These novel synthesized Thiosemicarbazides derivatives then carried out test for their anti-inflammatory action. Further design may prove an alternative and very useful and fruitful in the discovery of new anti-inflammatory activity in comparison to Aspirin as standard.

CONFLICTS OF INTEREST

There are no conflicts of interests.

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