Annexure-IX

University Grants Commission

Bahadur Shah Zafar Marg

New Delhi-110002

Final report of the work done on the project

1	Name and address of the principal investigator	Palwinder Singh
2	Name and address of the institution	Department of Chemistry, Guru Nanak Dev University
3	UGC approval No. and date	F. No. 32-202/2006 (SR) dated 24.03.2007
4	Date of implementation	01.04.2007
5	Tenure of the Project	01.04.2007 – 31.03.2010
6	Total grant allocated	Rs. 7,76,600
7	Total grant received	Rs. 7,06,000
8	Final expenditure	Rs. 7,05,124
9	Title of the project	Synthesis of α, β-diarylheterocycles with stereogenic centers: Search for phospholipase A_2 and COX-2 inhibitors
10	Objectives of the project	**i)** Diastereoselective synthesis of 5- and 6-membered saturated heterocycles with varied stereogenic centers **ii)** Synthesis of hybrid molecules possessing more than one heterocyclic moieties **iii)** Evaluation of COX-2 and PLA_2 inhibitory activities of synthesized molecules **iv)** Development of rational for binding and selectivity by both experimental results and dockings of these molecules in the active sites of COX-1,

		COX-2 and PLA_2 and selection of lead molecules **v)** Enantioselective synthesis of lead molecules and their derivatives and their evaluation as COX-2 and PLA_2 inhibitors.
11	Whether objectives were achieved (Give details)	Yes, Annexure-A
12	Achievements from the project	Annexure-A
13	Summary of the findings (In 500 words)	Annexure-B
14	Contribution to the society (Give detail)	New categories of compounds with excellent COX-2 inhibitory activities are developed which could be further refined to have drug like properties and therefore could led to new anti-inflammatory drug/s.
15	Whether any Ph.D. enrolled/produced out of the project	Produced - 1
16	No. of publications out of the project	04 (reprints attached) Appendix-C

(Principal investigator) (Registrar)

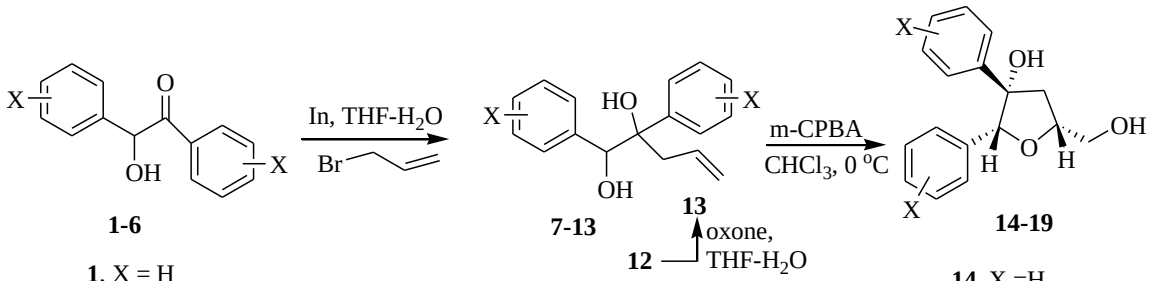
Annexure-A

(Achievements from the project)

As per the objectives of the project, a stepwise description of the work done in this project has been given below:

1. Synthesis of 5-membered oxygen containing heterocycles:

Highly stereoselective synthesis of the target molecules (**14**-**19**) was achieved through the allylation of benzoins (**1**-**6**) followed by the *m*-CPBA mediated cyclizations of homoallylic alcohols (**7**-**11** and **13**) (scheme 1).



1, X = H
2, X = 4-Cl
3, X = 2-Cl
4, X = 4-F
5, X = 4-OMe
6, X = 4-SMe

7, X = H
8, X = 4-Cl
9, X = 2-Cl
10, X = 4-F
11, X = 4-OMe
12, X = 4-SMe
13, X = 4-SO_2Me

14, X =H
15, X = 4-Cl
16, X = 2-Cl
17, X = 4-F
18, X = 4-OMe
19, X = 4-SO_2Me

Scheme 1

The stereochemistry at the various asymmetric centers of molecules **14**-**19** was established on the basis of NOE experiments and the X-ray crystal structure of compound **14** (figure 1). The observation of NOE between 2-H and 5-H is confirmed from the syn-placement of these two hydrogens in the X-ray structure of compound **14**.

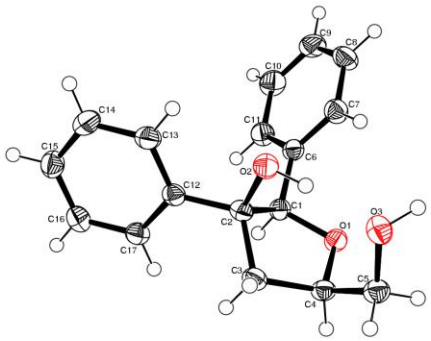
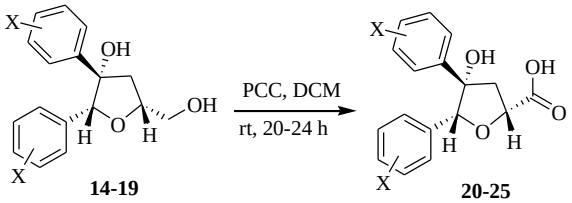
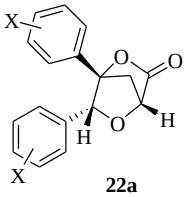


Figure 1. The ORTEP diagram of compound **14**

In order to introduce a carboxyl group at C-5 of tetrahydrofuran (designed on the basis of dockings of molecules in the active sites of COX-1 and COX-2), compounds **14-19** were subjected to PCC mediated oxidations. Treatment of compounds **14-19** with PCC in dichloromethane provided the corresponding tetrahydrofurans **20-25** (scheme 2). However, the X-ray structure of compound **22** shows the presence of a bis-lactone (**22a**) which might have formed by *in-situ* cyclization of compound **22**.



14-19 **20-25**



22a

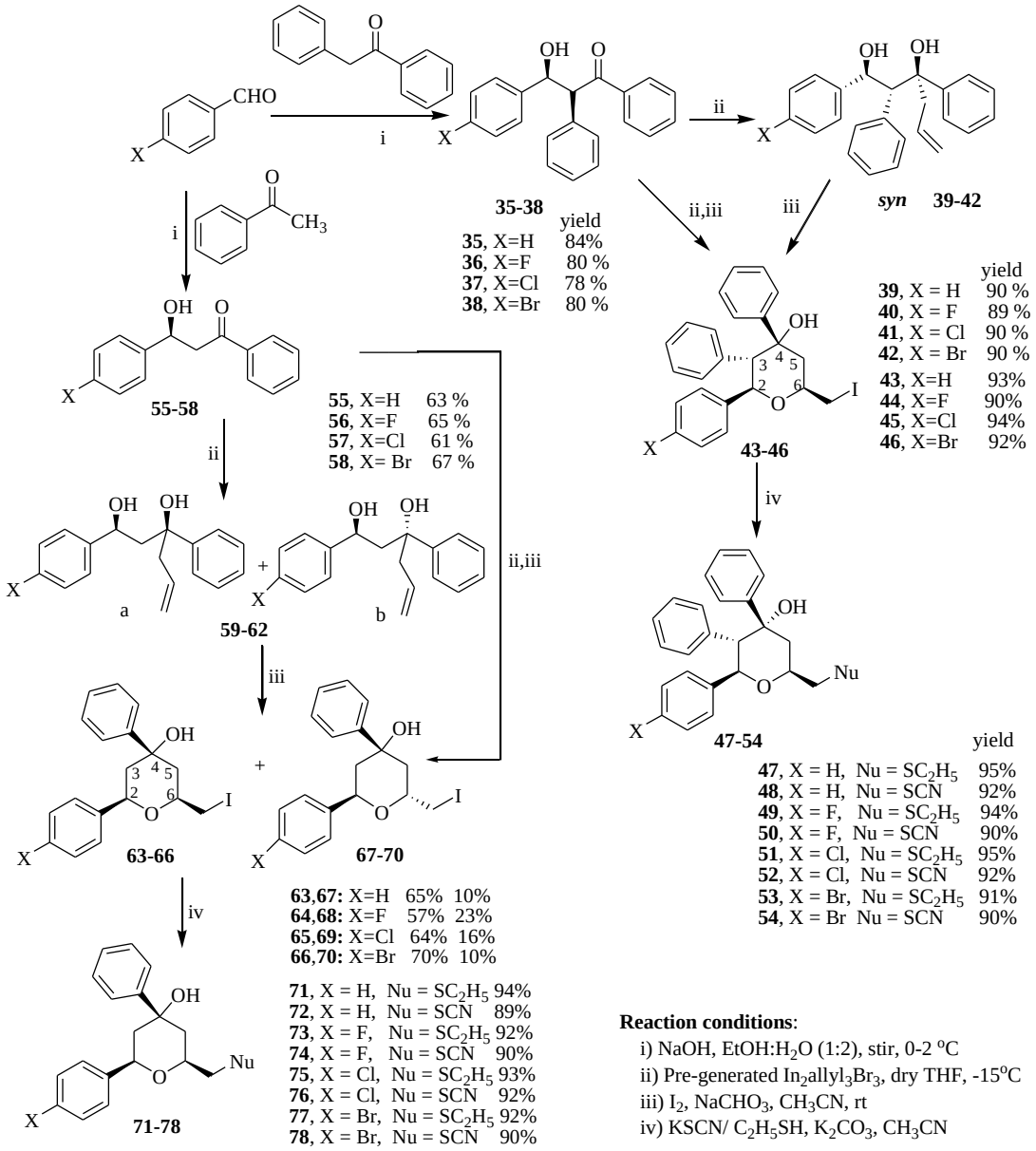
20, X =H
21, X = 4-Cl
22, X = 2-Cl
23, X = 4-F
24, X = 4-OMe
25, X = 4-SO_2Me

Scheme 2

Therefore, the tetrahydrofuran based molecules, designed on the basis of molecular dockings in the active sites of COX-1 and COX-2, have been synthesized in appreciable yields by the indium mediated allylations of benzoins followed by cyclizations with *m*-CPBA.

2. Synthesis of 6-membered oxygen containing heterocycles:

Tetrahdropyrans have been synthesized through allylation-iodocyclization of β-hydroxy ketones. It is pertinent to mention here that β-hydroxy ketones were synthesized by aldol condensation carried out at 0 ^{o}C while high diatereoselectivity of allylation of β-hydroxy ketones were achieved at -15±1 ^{o}C (scheme 3). The aldols **55-58** obtained by the reactions of benzaldehyde with acetophenone after allylation and iodocyclization provided tetrahydropyrans **63-70** carrying two phenyl rings. Similarly the aldols **35-38** obtained from the reactions of benzaldehydes with deoxybenzoin, after allylation and iodocyclization gave tetrahydropyrans **43-46** substituted with three phenyl rings. Tetrahydropyrans **43-46** and **63-70** were further derivatized by the replacement of iodo group with other nucleophiles.



Scheme 3

3. Evaluation of COX-2 inhibitory activities of synthesized molecules

THPs **71-78** exhibit significant COX-2 inhibitory activities with their IC_{50} values 0.9-5.5 nM. However, the compounds **77** and **78** with Br at one of the two aryl rings exhibited slightly less COX-2 inhibitory activities (IC_{50} = 4-5.5 nM) in comparison to the analogous compounds **71-76** (IC_{50} = 0.9-3.5 nM). Interesting results were observed for the COX-1/2 inhibitory activities of tri-aryl substituted compounds **47-54**. All these compounds exhibited very poor inhibition of COX-1 (IC_{50} = 10-64 μM, Table 1, Table

S3) while their COX-2 inhibitory activities (IC_{50} = 0.57-4.0 nM) were comparable or better than compounds **71-78**. Although COX-2 inhibitory activities of compounds **47-54** were similar to those exhibited by compounds **71-78** but better selectivity for COX-2 over COX-1 [**71-78** SI = 50-1900, **47-54** SI = 3200-44000] was observed. Compounds **49-52** with F and Cl substituent at one of the three phenyl rings showed better inhibition of COX-2 in comparison to the analogues with H and Br present on the respective phenyl ring. It seems that with the increase in the number of phenyl rings on THP, the effect of π-π interactions predominates over other interactions and hence the other variations in the compound (substituent at phenyl ring and *C*-6 of THP) have little or no effect on COX-2 inhibitory activities. The COX-2 inhibitory activity and the selectivity for COX-2 over COX-1 for all these compounds were better than the known COX-2 inhibitors *viz*. celecoxib and rofecoxib. Therefore, as per the design of these molecules, based upon their size, they exhibited moderate to high COX-2 inhibitory activity as well as the selectivity for COX-2 over COX-1 and they are worth to undergo further investigations.

Table 1. 50% inhibitory concentrations (IC_{50}) of compounds **71-78** and **47-54** for COX-1 and COX-2.

Compd.	IC_{50} (nM)[a]		Selectivity index (SI)[b]
	COX-2	COX-1	
71	2.07	2932	1416
72	1.47	2788	1896
73	2.05	1738	847
74	3.57	488	136
75	1.82	413	226
76	0.9	664	737
77	5.49	562	102

78	4.01	209	52
47	1.72	13880	8069
48	1.55	63990	41283
49	1.28	~46990	~36710
50	1.11	28850	25990
51	0.57	10700	18771
52	0.65	28650	44076
53	2.8	23800	8500
54	4.03	12960	3215
celecoxib	70	33100	473
rofecoxib	500	>100000	>200

4. Enantioselective synthesis of tetrahydropyrans

Enantioselective synthesis of polysubstituted tetrahydropyrans has been achieved by the allylations of enantiomerically enriched β-hydroxy ketones followed by diastereoselective iodocyclizations. β-Hydroxy ketones have been procured stereoselectively through the reactions of appropriate aldehydes and ketones mediated by small organic molecules *viz*. proline and its derivatives. Here, improved over the reported procedures for the synthesis of 4-hydroxy-4-(4-nitrophenyl)-butan-2-one (**2**, R=NO_2), it has been synthesized (72%, $[\alpha]_D$ = +44^o and 72 % ee) by the reaction of 4-nitro-benzaldehyde with acetone (as solvent) using proline as catalyst (scheme 4). Under the same reaction conditions, the treatment of other benzaldehydes with acetone provided the corresponding β-hydroxy ketones in comparable yields and enantioselectivities as reported using DMSO/acetone as the solvent and 'proline derivatives' as the catalysts.

The *R*-configuration at the chiral center of these hydroxy ketones have been confirmed from the X-ray structure of **2** (R=NO_2) (figure 2).

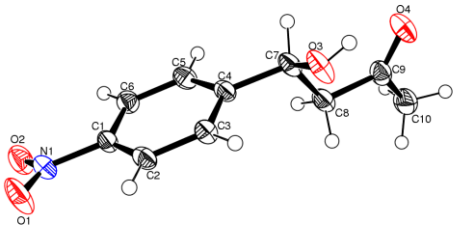
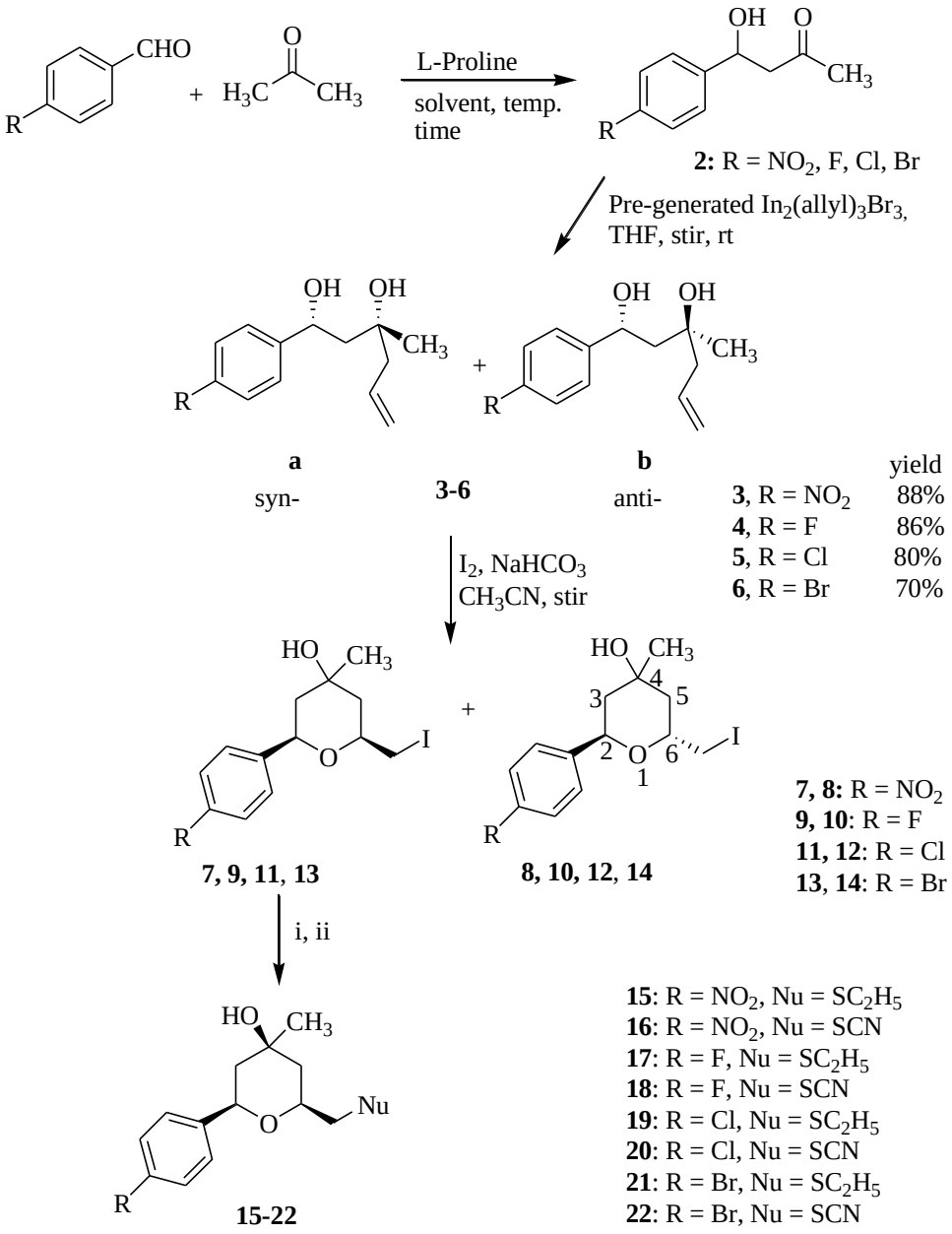


Figure 2. ORTEP diagram of compound **2** (R = NO_2)

To the cooled solution of the reagent $In_2(allyl)_3Br_3$, generated by refluxing the mixture of allyl bromide (0.5 mmol) and indium metal (0.4 mmol) in dry THF, was added **2** (R = NO_2, 1 equiv). Stirring the reaction mixture at room temperature, after usual work up and column chromatography provided a mixture of two diastereomers **3a** and **3b** (88%, M^+ m/z 251) which in ^1H NMR spectrum clearly shows two sets of signals in the ratio 7:3 (scheme 4). Under the same reaction conditions, the treatment of **2** (R = F, Cl, Br) with pre-generated reagent $In_2(allyl)_3Br_3$, gave the corresponding compounds **4**, **5** and **6** as a mixture of two diastereomers (a and b) in the ratio 5:4, 2:1 and 7:3 respectively (scheme 4). Treatment of diastereomeric mixture of **3** with iodine in dry CH_3CN using $NaHCO_3$ provided a mixture of two compounds (2:1, ^1H NMR spectrum) which after purification with column chromatography have been identified as compounds **7** (40%, $[\alpha]_D$ = +43.3^o) and **8** (20%, $[\alpha]_D$ = +41^o) (scheme 4). Similar reactions of **4**, **5** and **6** provided compounds **9** (35%, $[\alpha]_D$ = +40^o), **10** (30%, $[\alpha]_D$ = +35^o); **11** (35%, $[\alpha]_D$ = +47^o), **12** (30%, $[\alpha]_D$ = +48^o) and **13** (32%, $[\alpha]_D$ = +41.2^o), **14** (21%, $[\alpha]_D$ =+40^o) respectively (scheme 4).

The relative stereochemistries at the various asymmetric carbons of compounds **7**-**14** have been ascertained on the basis of NOE experiments (figure 3) and X-ray structure of **13** (figure 4). The observation of NOE between 2-H and 6-H in the case of compounds **7**, **9**, **11** and **13** indicates the syn orientation of these hydrogens. The X-ray structure of **13** (figure 4) shows the equatorial orientation of phenyl ring, CH_2I and OH groups and

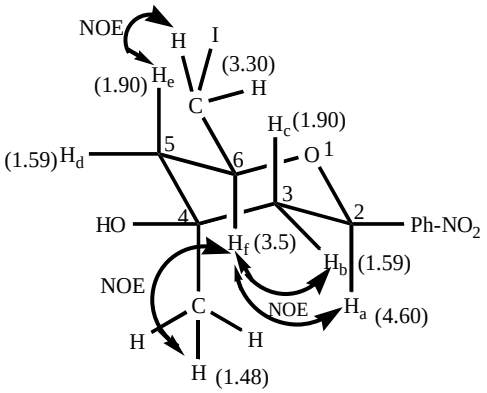


	yield	dr(a:b)
3, R = NO_2	88%	70: 30
4, R = F	86%	56: 44
5, R = Cl	80%	67: 33
6, R = Br	70%	70: 30

Reagents and reaction conditons: i) C_2H_5SH, K_2CO_3, CH_3CN, stir, rt;
ii) KSCN, K_2CO_3, CH_3CN, stir, rt

Scheme 4

supports the stereochemistries observed for these compounds on the basis of NMR experiments. Compounds **15**-**22** (analogues of **7**, **9**, **11** and **13**) with stereochemistries at various asymmetric carbons as shown in figure 4 were used for docking studies.



$J_{a\text{-}b}$ = 2.1 Hz, $J_{a\text{-}c}$ = 11.7 Hz, $J_{d\text{-}f}$ = 2.1 Hz, $J_{e\text{-}f}$ = 11.4 Hz

Figure 3. Orientation of the groups at each carbon of **7** as depicted from ^{1}H decoupling and NOE experiments. ^{1}H Chemical shifts are given in brackets.

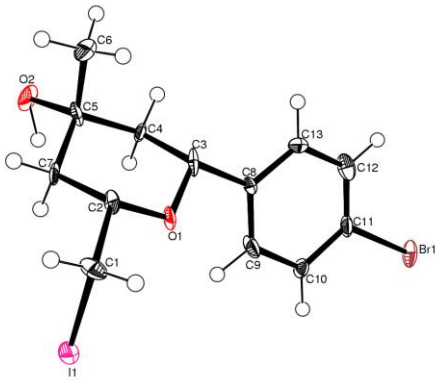


Figure 4. ORTEP diagram of **13**

It has been found that the groups like $CH_2SCH_2CH_3$, CH_2SCN etc., when present at 5-membered cyclic template, are suitable for interacting with the guanidine moiety of R120, an amino acid present in the active site of COX-2. In order to introduce such groups on tetrahydropyrans, equimolar quantities of **7** / **9**/ **11**/ **13** and C_2H_5SH / KSCN were stirred in CH_3CN using K_2CO_3 as base which provided the respective compounds **15-22** (scheme 4).

Therefore, starting from β-hydroxy ketones, following a two step synthetic approach *viz*. allylation and iodocyclization, polysubstituted tetrahydropyrans have been procured in moderate to high yields.

Bio-evaluations as COX-2 inhibitors

In-vitro evaluations of these compounds as COX-1 and COX-2 inhibitors were carried out using 96 well plate on the basis of production of prostaglandins by COX-1 and COX-2 enzymes in the presence of inhibitors in comparison to the control experiments. Compounds **15-22** (except **20**) (scheme 4) were evaluated in duplicate at 10^{-5} M and 10^{-6} M concentrations for COX-2 inhibition and 10^{-5} M concentration for COX-1 inhibition (table 2). For compounds **15-22**, almost no difference in the COX-2 inhibitory activities between compounds **15** and **16**; **17** and **18**; **21** and **22** has been observed which indicates

that $CH_2SC_2H_5$ and SCN groups may contribute equally towards the activity of these compounds. It was also found during the docking studies that the S atom of both these substituents approaches to R120 in the active site of COX-2. Compounds **17** and **18** with F on the aryl ring show considerably higher inhibition of COX-2 (IC_{50} ~1μM) as compare to other compounds with NO_2, Cl and Br substituted aryl rings. Moreover, **17** and **18** exhibit lower inhibition of COX-1 in comparison to ibuprofen (a non-selective COX-1/2 inhibitor). Therefore, these investigations identify compounds **17** and **18** for further refinement to develop as COX-1/2 inhibitors.

Table 2. *In-vitro* percentage inhibition of COX-1 and COX-2 by compounds **15-22** (scheme 4).

Compd	Percentage inhibition			IC_{50} (μM)	
	COX-2		COX-1	COX-2	COX-1
	10^{-5}M	10^{-6}M	10^{-5}M		
15	37	37	34	>10	>10
16	35	34	29	>10	>10
17	56	55	42	<1	>10
18	49	47	34	1	>10
19	34	30	34	>10	>10
20	nd	nd	nd	-	-
21	30	30	24	>10	>10
22	28	26	32	>10	>10
Ibuprofen[25]	76	87	96		
Aspirin (IC_{50})[26]				2.4	0.35

nd – not done

During the docking of compound **15** in the active site of COX-2 (figure 5), the nitro group present at the phenyl ring shows H-bonding with H90 residue and the S atom

present at C-6 substituent approaches R120 (the amino acid active during the metabolic phase of COX-2) at a distance of 2.7 Å. When **15** is docked in the active site of COX-1 (figure 6), the C-6 substituent ($CH_2SC_2H_5$) is placed in the hydrophobic sub-pocket of COX-1 active site constituted by Y385, W387, F381 and F518 residues. The nitrophenyl group present at C-2 of **15** is oriented towards the polar sub-pocket comprising R120, V116, Y355 residues. A similar placement of molecules **16**-**22** has been observed during their dockings in the active sites of COX-1 and COX-2.

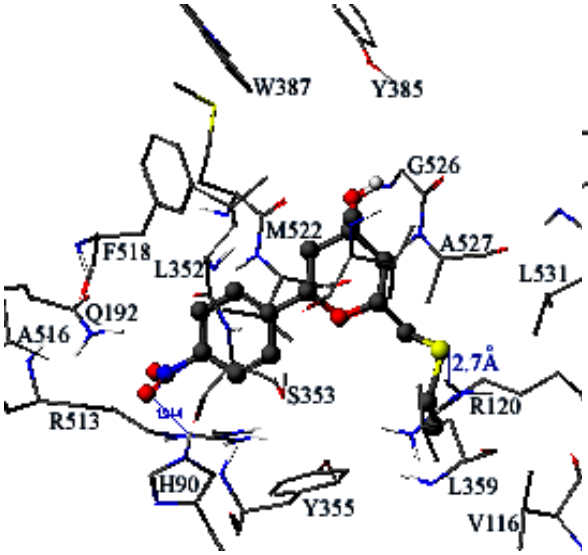


Figure 5. **15** (scheme 3) docked in the active site of COX-2. One of the oxygens of the nitro group of **15** forms H-bond with H90 while S atom present at C-6 substituent approached R120 at a distance of 2.7 Å.

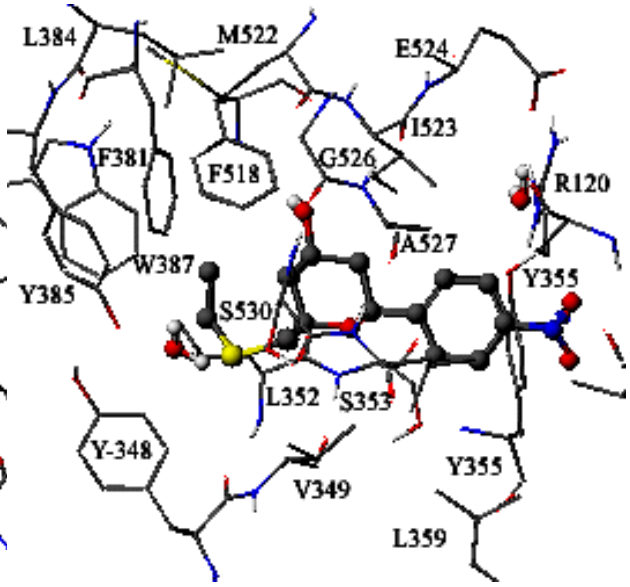


Figure 6. Compound **15** (scheme 3) docked in the active site of COX-1.

The optimum size and appropriate stereochemistry of these molecules allows them to enter the active sites of COX-1 as well as COX-2, thereby inhibiting both these enzymes. The close parallelism between the docking results and the experimental results could be helpful in further refinements of these molecules.

Quantitative structure activity relationship (QSAR) studies indicate the dependence of these biological results on the partition coefficient and total polar surface area of the molecules. On the basis of these preliminary results and QSAR studies, further refinement of these molecules is underway.

Annexure-B

(Summary of the findings)

Tetrahydrofurans and tetrahydropyrans, substituted with aryl rings and other groups, were synthesized and evaluated for in-vitro COX-1, COX-2 inhibitory activities. A versatile synthetic methodology, starting from the allylation of α–hydroxy ketones and β–hydroxy ketones followed by iodocyclization has been developed for tetrahydrofurans and tetrahydropyrans respectively. An excellent structure activity relationship was drawn for the COX inhibitory activities of these molecules. In parallel with the difference in the size of active site of COX-2 from COX-1, with the increase in the size of tetrahydropyrans from tetrahydrofurans, the COX-2 inhibitory activities as well as the selectivity for COX-2 over COX-1 increases.

In the tetrahydrofuran series of compounds, the presence of aryl rings on the vicinal carbons along with the presence of CH_2SCN or $CH_2SCH_2CH_3$ on tetrahydrofuran ring is essential for COX-2 inhibitory activities (Chart 1).

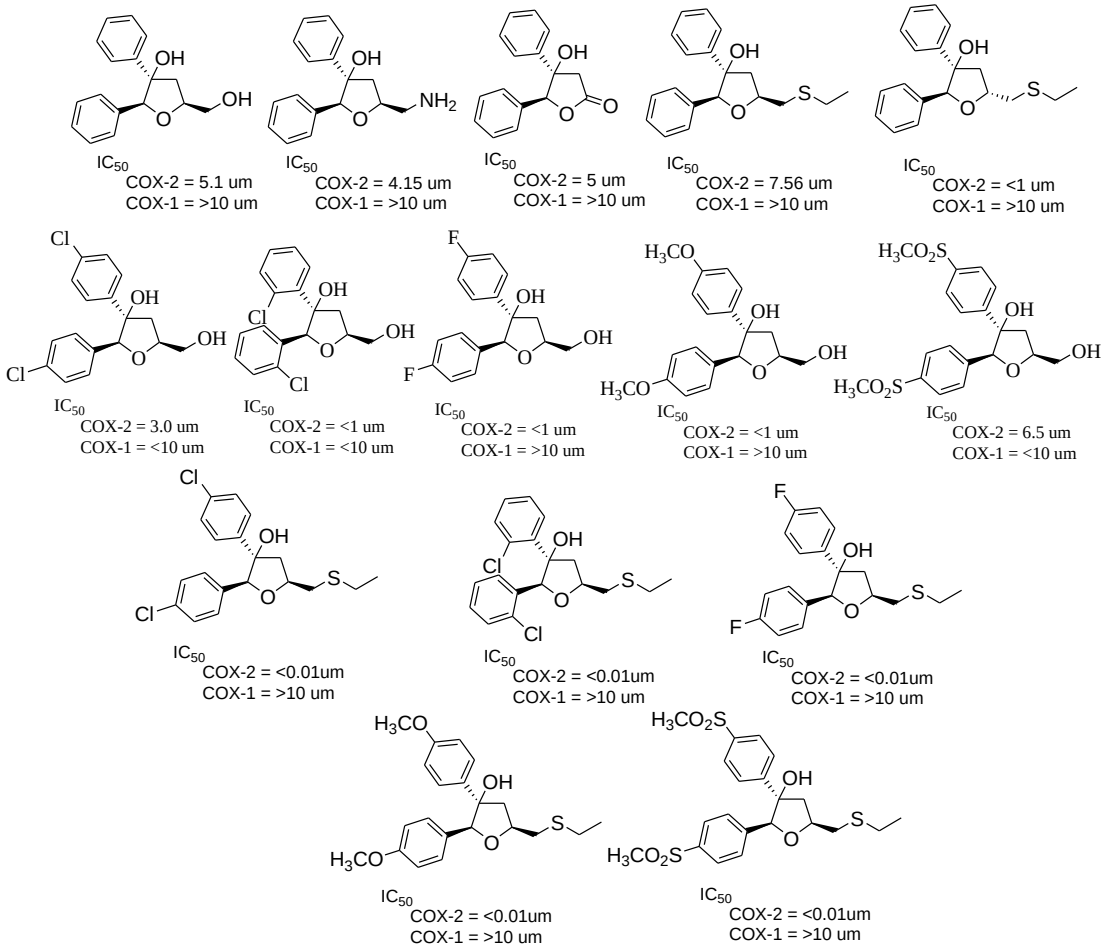
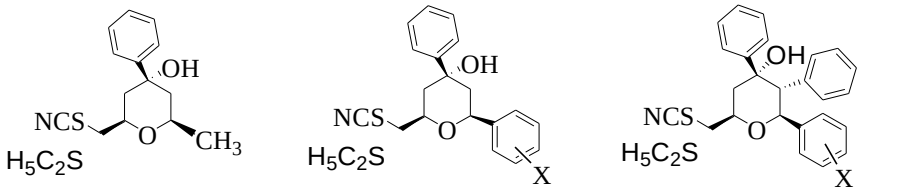


Chart 1

For tetrahydropyran based molecules, it has been observed that tetrahydropyrans with one phenyl ring are moderate inhibitors of both COX-1 and COX-2, tetrahydropyrans with two aryl rings are showing high inhibition for COX-1 and COX-2 while triaryl substituted tetrahydropyrans are highly selective for COX-2 (Chart 2).



moderate inhibitor of COX-1 and COX-2

good inhibitors of COX-1 and COX-2

high selectivity for COX-2 over COX-1

Chart 2

Therefore, in accordance with the difference in the sizes of the active sites of COX-1 and COX-2, a control over the size/volume of the ligand could help in the design of moderate/selective inhibitors of COX-1/2. The interactions of tetrahydrofurans and tetrahydropyrans with the amino acids of the active sites of COX-1 and COX-2 are also explored with docking studies. Some of the tetrahydropyrans are under investigations at NCI, NIH, USA for anticancer activities.

Appendix-C

(Details of publication resulting from the project work)

S. No.	Title	Authors	Journal
1.	2,3,5-Substituted tetrahydrofurans as cancer chemopreventives. Part 1: Synthesis and anti-cancer activities of 5-hydroxymethyl-2,3-diaryltetrahydrofuran-3-ols	Palwinder Singh, Anu Mittal, Subodh Kumar	**Bioorg. Med. Chem. 2007**, *15*, 3990-3996.
2.	2,3,5-Substituted tetrahydrofurans: COX-2 inhibitory activities of 5-hydroxymethyl-/carboxyl-2,3-diaryltetrahydrofuran-3-ols	Palwinder Singh, Anu Mittal, Satwinderjeet Kaur	**Eur. J. Med. Chem. 2008**, 43, 2792-2799.
3.	Design, synthesis and evaluation of tetrahydropyran based COX-1/-2 inhibitors	Palwinder Singh, Atul Bhardwaj, Satwinderjeet Kaur, Subodh Kumar	**Eur. J. Med. Chem. 2009,** 44, 1278-1287
4	Mono-/, di-/, tri-aryl substituted tetrahydropyrans as cyclooxygenase-2 and tumor growth inhibitors. Synthesis and biological evaluation	Palwinder Singh and Atul Bhardwaj	**J. Med. Chem. 2010,** 53, 000 **DOI:** 10.1021/jm1001327