Saudi Journal of Biological Sciences 27 (2020) 1208–1216

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Spiroindolone analogues bearing benzofuran moiety as a selective cyclooxygenase COX-1 with TNF-α and IL-6 inhibitors

Mezna Saleh Altowyan [a], Assem Barakat [b,c,*], Abdullah Mohammed Al-Majid [b], H.A. Al-Ghulikah [a]

[a] Department of Chemistry, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia
[b] Department of Chemistry, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia
[c] Department of Chemistry, Faculty of Science, Alexandria University, P.O. Box 426, Ibrahimia, Alexandria 21321, Egypt

ARTICLE INFO

Article history:
Received 17 December 2019
Revised 12 February 2020
Accepted 17 February 2020
Available online 26 February 2020

Keywords:
Spirooxindole
Lipid metabolic enzymes
Pro-inflammatory cytokines
COX-1
COX-2
IL-6
TNF-α

ABSTRACT

To design and discover a new compound can used as a COX with TNF-α and IL-6 inhibitors is highly challenge. A series of spiroindolone-bearing benzofuran moieties were resynthesized from the chalcone-based benzo[*b*]furan with substituted isatin, and amino acids. The requisite spiroindolone analogues were tested for their potential inhibitory activities against lipid metabolizing enzymes such as cyclooxygenase COX-1, COX-2, and the release of pro-inflammatory cytokines interleukin IL-6, and tumor necrosis factor TNF-α. Among the tested compounds, **5a, 5c, 5h, 5i, 5l**, and **5p** exhibited COX-1 inhibitor selectively with percent of inhibition 40.81–83.4% and IC_{50} values ranging from 20.42 μM to 38.24 μM. In addition, all the synthesized target compounds possessed lipopolysaccharide-induced TNF-α, and IL-6 expression with a varying degree of COX-1 inhibition. Compounds **5d**, **5e**, **5f**, **5g**, and **5k** markedly inhibited TNF-α, and IL-6 release in WI-38 fibroblast cells. Molecular docking of the most effective and highly selective compounds were investigated and shown important binding mechanisms which could affect pro-inflammatory enzymes and cytokines via the inhibition of COX-1, COX-2, IL-6, and TNF-α.

1. Introduction

Several factors affect the development of cancer including the combination of environmental, and host factors. Inflammation has been linked and increased the risk for development different type of tumors such as gastric mucosal lymphoma, colon cancer, gastric cancer, and prostate cancer. Many factors lead to chronic inflammation such as microbial infections (e.g., *Helicobacter pylori* infection), autoimmune diseases (e.g., inflammatory bowel disease), inflammatory conditions (e.g., prostatitis), chemical compounds (e.g., ROS), or physical injuries (e.g., exposure to UV) (Balkwill and Mantovani, 2001; Koehne and Dubois, 2004; Chan et al., 2001; Mantovani et al., 2008).

There are two cyclooxygenase COX enzymes, namely cyclooxygenase-1 COX-1, and cyclooxygenase-2 COX-2. The cytoprotection process in the gastrointestinal tract and normal renal function is regulated by the constitutive isozyme COX-1. COX-2 is a proactive, and short-lived enzyme; its expression occurs as a pro-inflammatory response. COX-2 is involved in prostaglandin biosynthesis in inflammatory cells of the central nervous system (Marnett et al., 1999; Herschman, 1996). The activation of such enzymes stimulates intracellular signals (i.e., NFκB (Takeda et al., 2003; Ben-Neriah and Karin, 2011), p38, or MAPKs (Dhanasekaran and Johnson, 2007), which modify pro-inflammatory cytokine expression e.g., interleukin 1 beta (IL1β) (Church et al., 2008), interleukin 6 (IL6) (Trikha et al., 2003), and tumor necrosis factor alpha (TNF-α) (Tracey et al., 1994). These signals combined with cell adhesion proteins and chemokines leads to the stimulation and activation of immune cells. Therefore, many researchers focus on developing novel and selective inhibitors of pro-inflammatory enzymes and cytokines.

Oxindole derivatives are known as anti-tumor agents due to their tyrosine kinase inhibitory property. Additionally, the oxindole scaffold exhibits promising structural features and a good bioactivity profile including antiviral, antimicrobial, and local anesthetic properties (Galliford and Scheidt, 2007; Marti and

* Corresponding author at: Department of Chemistry, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.
E-mail address: ambarakat@ksu.edu.sa (A. Barakat).
Peer review under responsibility of King Saud University.

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https://doi.org/10.1016/j.sjbs.2020.02.010
1319-562X/

Carreira, 2003; Trost and Brennan 2009; Ding et al., 2005; Padwa and Pearson, 2002; Wade, 1991). Therefore, these compounds may further the design of novel lead compounds with anti-inflammatory, and anticancer activities. Recently, one such compound, **JP-8g**, exhibited high efficacy against cancer, and inflammation (Sun et al., 2014, Sun et al., 2015). Among synthetic compounds, oxindole gained attention as a potential anticancer drug. In this regard, compounds **SPX-F** (Jiang et al., 2010), and **4d** (Barakat et al., 2017, Barakat et al., 2019) have exhibited efficacy for MDM2–p53 protein–protein interaction inhibitor and p53 reactivation in cancer cells (Fig. 1).

Benzo[*b*]furan is an interesting pharmacophore which exists in several synthetic and natural hits and has pharmaceutical applications (Dawood, 2013). Some publications report the benzo[*b*]furan moiety to inhibit activities of human immunodeficiency virus (HIV) and hepatitis C virus (HVC). In addition, it also has possessed as antioxidant, cytotoxic, anti-inflammatory (e.g. ailanthoidol), antitumor, antimicrobial, antiplasmodial and, antitubercular activities (Dawood, 2013) (Fig. 1).

A derivative of celecoxib based a benzo[*b*]furan moiety was reported to demonstrate selective activity against COX-2 (Hassan et al., 2014). In addition, new molecules containing rhodanine and benzofuran scaffolds were designed, synthesized, and reported to exhibit dual COX-2, and 5-LOX inhibitory potential. Recent patent survey reported as a review focused on the benzofuran inhibitors (El-Miligy et al., 2017).

In this study, a series of compounds having the oxindole, and benzo[*b*]furan scaffolds were resynthesized according to Altowyan et al. (2019). These highly functionalized complex molecules, with different pharmacophores, may have potential applications in diverse, and economically relevant areas.

2. Materials and methods

The target compounds were resynthesized in accordance with a previous article (Altowyan et al., 2019). Additionally, all enzymatic assays and the molecular docking studies details have been provided in the supplementary information.

3. Results and discussion

3.1. Preparation of *5a-r*

A number of spirooxindole benzo[*b*]furan scaffolds were resynthesized using the 1,3-dipolar cycloaddition reaction method. As shown in Fig. 2, the synthetic pathway consist of the reaction of chalcone **2a-f** with dicarbonyl compounds (isatin, 5-chloroisatin, and 5-bromoisatin **3a-c**) and amino acids including (*S*)-thiazolidine-4-carboxylic acid or (2*S*,3a*S*,7a*S*)-octahydro-1*H*-indole-2-carboxylic acid. In this manner, a number of spirooxindole based benzo[*b*]furan scaffolds (**5a-r)** were obtained in high analytical purity with complete stereoselective reaction (Scheme 1). The synthetic methodology was carried out under mild alcoholic conditions, which is considered an eco-friendly approach. The general reaction is shown below, as variations of all three reactants are possible. The mechanism of cyclization is in accordance to previously established methods (Islam et al., 2019; Barakat et al., 2018, Lotfy et al., 2018, Lotfy et al., 2019).

3.2. In vitro biological activity evaluation

3.2.1. COX-1 and COX-2 inhibitors

The COX inhibitory activity of resynthesized target compounds were screened *in vitro* for the two isoforms of COX (Abdellatif et al., 2018). COX (COX-1, and COX-2) at 40 μg/mL and the results obtained were shown in Table 1. The IC_{50} values were determined, and the data are summarized in Table 1. The data in Table 1 suggested that compounds **5a, 5c, 5h, 5i, 5l**, and **5p** possessed powerful COX-1 inhibitory abilities ranging from 40.81−83.4%. On the other hand, the inhibition of COX-2 was weaker, and was shown in the range of 1.11–20.56%. Indomethacin, acetyl-keto boswellic acid (AKBA), acetyl-*β*-boswellic acid, acetyl-*α*-boswellic acid, and *β*-boswellic acid were used as positive controls for COX-1. Celecoxib was used as a positive control for COX-2. In the case of COX-1 inhibition, the IC_{50} values recorded for the most active compounds were in the range of 20.42–38 μM; these included **5a, 5c, 5h, 5i, 5l**, and **5p**. Compound **5a** (IC_{50} = 20.42 μM) exhibited the strongest inhibition of COX-1 between these series. It was

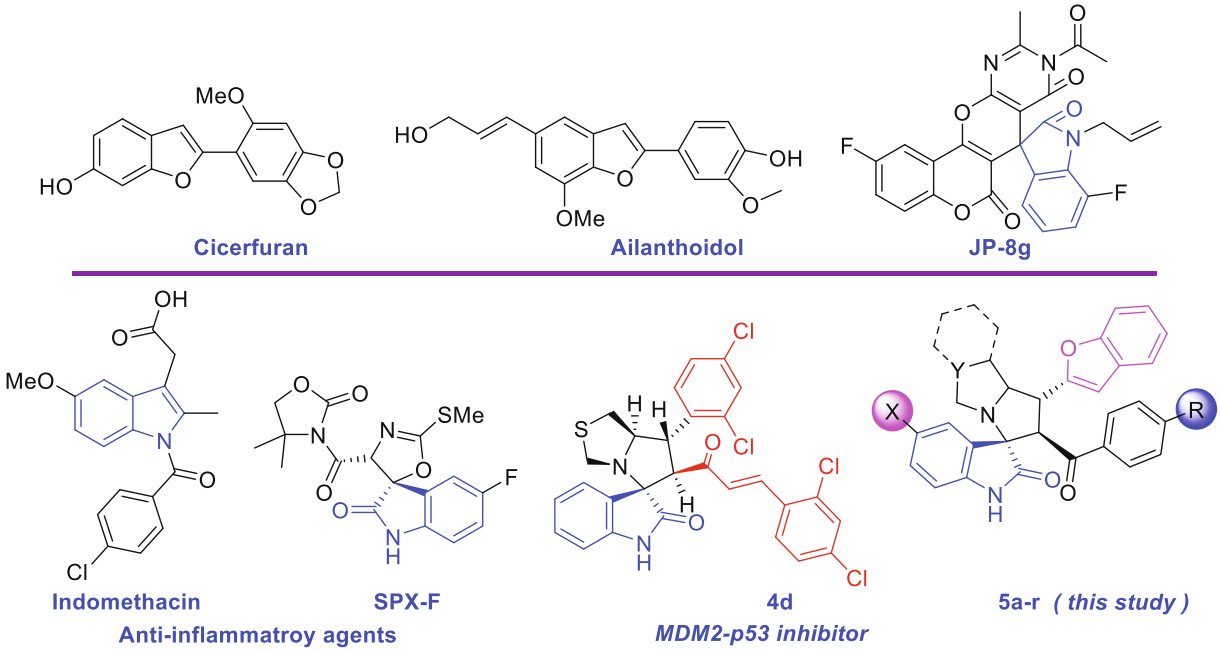


Fig. 1. Representative examples of spirooxindoles and benzo[*b*]furan scaffolds.

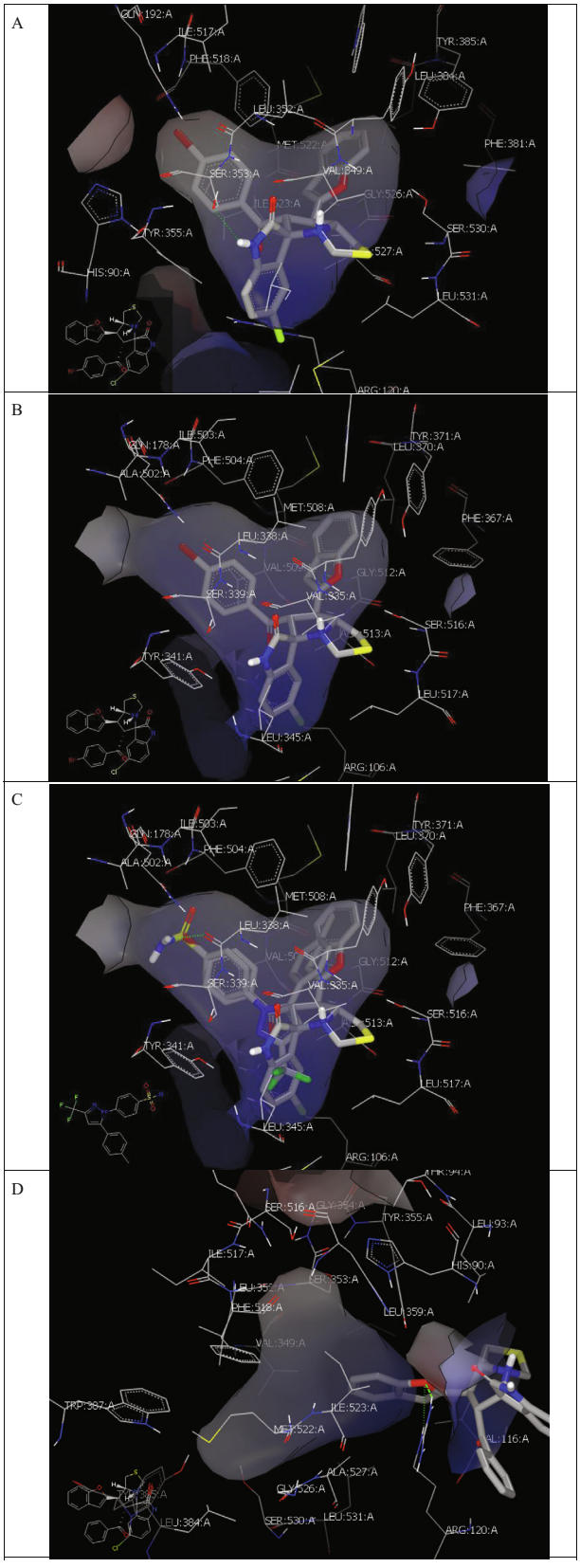


Fig. 2. (**A**) Hydrophobic interactions embed compound **5i** inside COX-1 receptor (ID: 5WBE). (**B**) Hydrophobic interactions embed compound **5i** inside COX-2 receptor (ID: 3LN1). (**C**) Compound **5i** is overlaid with celecoxib inside COX-2 receptor (ID: 3LN1). (**D**) Compound **5a** with (ID: 5WBE) docked outside the receptor through formation of two HBs with ARG 120.

observed that the analogs were less selective against COX-2 than against COX-1.

3.2.2. Inhibition of TNF-α and IL-6 release in LPS-stimulated cells

The inhibitory activity of the resynthesized compounds was examined against LPS-induced TNF-α, and IL-6 in WI-38 fibroblast cells. All the examined compounds inhibited LPS-induced TNF-α, and IL-6 expression to different extents. Compounds **5d**, **5e**, **5f**, **5g**, and **5k** were the most effective among this compounds (Table 1).

3.3. Docking study

The studied compounds were subjected to molecular docking study to evaluate their activities as anti-inflammatory agents by analyzing their ability to inhibit COX-1 (PDB: ID 5wbe (Loll et al., 1996), and PDB: ID 1pgf (Wang et al., 2010), COX-2 (PDB: ID 3ln1) (He et al., 2005), and TNF-α (PDB: ID 2az5) (Wang et al., 2010). Moreover, COX-1/COX-2 selectivity was explained.

The docking mode of final compounds with COX-1(PDB: ID 5wbe) (Cingolani et al., 2017) showed that these compounds could be classified into two groups: Group (a) of compounds which interact with the protein inside the specific receptor of standards such as compound **5i** (Fig. 2A–C), and the second group (b) of compounds where the standard ligand docked outside the receptor through formation of hydrogen bonding (HB) interaction such as compound **5a** (Fig. 2D).

Compound **5i** interacts with key residues of COX-1, and COX-2 receptors *via* hydrophobic interactions through the benzofuran, indole, and *p*-bromophenyl moieties, Fig. 2A, and B respectively. Compound **5i** is overlaid with celecoxib inside the COX-2 receptor (ID: 3LN1). Biological studies revealed that both compounds (**5i** and **5p**) are non-selective. This were clarified by their ability to adapt inside the receptors of both COX-1 and COX-2 using the same binding mode (hydrophobic interactions) (Fig. 2A–D). Compound **5a** from the group (b) interacts with the receptor by formation of two HBs with Arg:120A via the oxygen of benzofuran moiety. This strong interaction helps to understand their selectivity.

The docking of the most active compounds with TNF-α (PDB: ID 2az5) was then studied. The standard ligand docked inside the receptor in a U-shape through the formation of hydrophobic interactions, Fig. 3A.

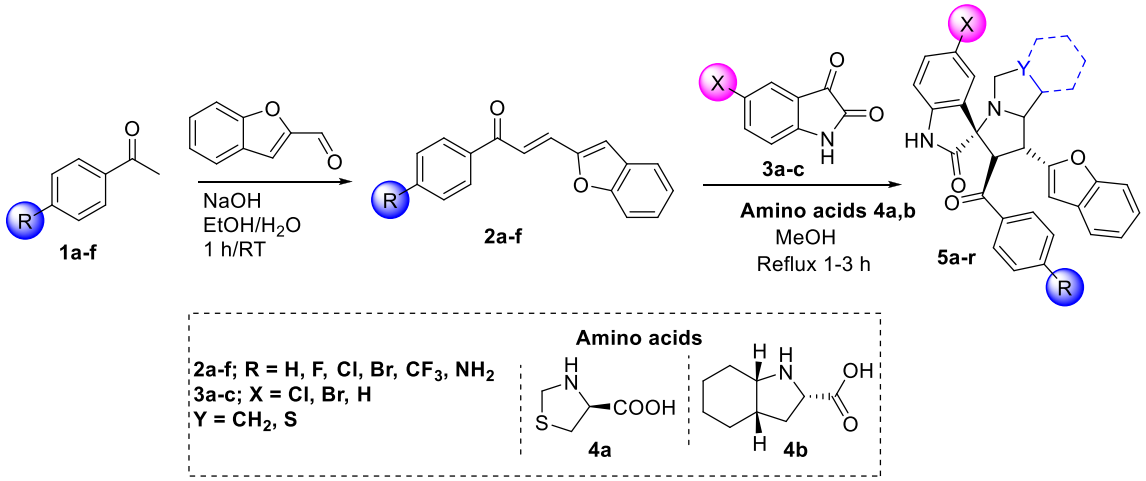
Compound **5a** docked inside the receptor through the formation of hydrophobic-hydrophobic interactions. Moreover, it overlaid onto the standard drug via similar interactions with the receptor cleft, Fig. 3(B, C).

3.4. Structure activity relationship

The structure activity relationship (SAR) of the studied compounds, and their biological activities with COX-1, COX-2, and the release of IL-6, and TNF-α were evaluated. The data presented in Tables 1, and Fig. 4 demonstrate that:

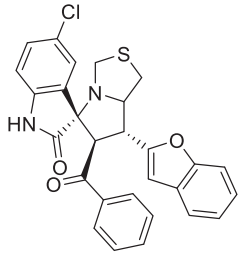
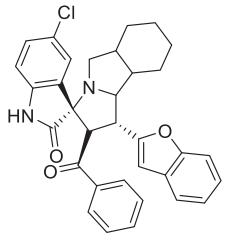
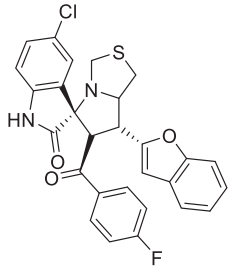
(i) Compounds **5a, 5c, 5h, 5i, 5l**, and **5p** which were synthesized based on the thiazole amino acid rather than fused cyclohexane-proline, lead to better inhibition of COX-1, COX-2, IL-6, and TNF-α. This could be due to the geometry of the chair form, and its role in positioning benzofuran inside the receptor.

(ii) Compound **5a,** with a chlorine atom on the oxindole moiety, and an un-substituted benzene on the benzoyl nucleus, showed the best, and selective results with 57.38% inhibition



Scheme 1. The resynthesized compounds **5a-r.**

Table 1
Results of percentage of inhibition of COX-1, and COX-2, IC_{50}, selectivity COX-1/CO-2; percentage of inhibition of IL-6 (pg/ml), and TNF-α of the synthesized spirooxindoles based on benzo[*b*]furan scaffold **5a-r**.

#	Compound	COX-1%	COX-2%	COX-1	COX-2	Selectivity[b]	IL-6 (pg/ml)%	TNF-α (pg/ml)%
				IC_{50} (μM ± SD)[a]		IC_{50} COX-1/IC_{50} COX-2		
1	 **5a**	57.38	11.24	20.42 ± 0.55	88.09 ± 1.78	23%	57.38	51.24
2	 **5b**	27.36	9.81	ND[c]	ND	ND	27.36	9.81
3	 **5c**	40.81	8.33	37 ± 1.54	76.17 ± 1.46	49%	59.81	48.33

(*continued on next page*)

Table 1 (*continued*)

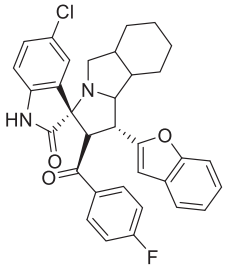
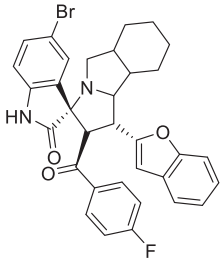
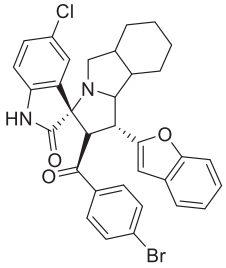
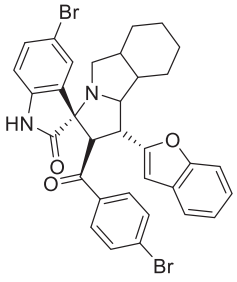
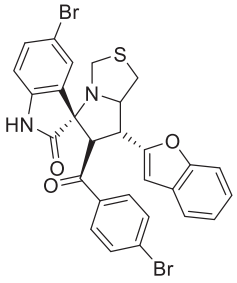
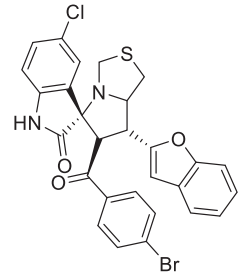
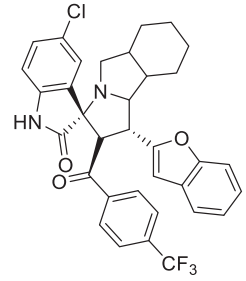
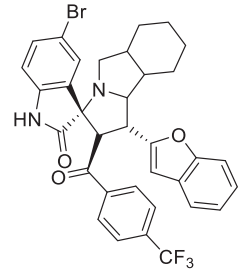
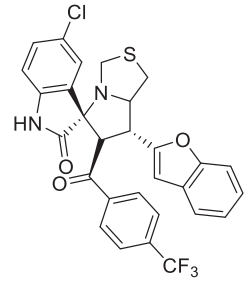
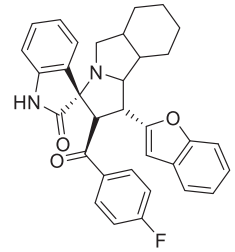
#	Compound	COX-1%	COX-2%	COX-1	COX-2	Selectivity[b]	IL-6 (pg/ml)%	TNF-α (pg/ml)%
				IC_{50} (μM ± SD)[a]		IC_{50} COX-1/IC_{50} COX-2		
4	 **5d**	19.81	7.77	ND	ND	ND	76.81	67.77
5	 **5e**	9.43	1.8	ND	ND	ND	82.43	71.8
6	 **5f**	32.08	5.92	ND	ND	ND	63.08	55.92
7	 **5g**	19.81	2.76	ND	ND	ND	81.81	78.76
8	 **5h**	41.70	9.8	35.70 ± 0.51	84.66 ± 1.16	42%	38.70	49.8

Table 1 (*continued*)

#	Compound	COX-1%	COX-2%	COX-1	COX-2	Selectivity[b]	IL-6 (pg/ml)%	TNF-α (pg/ml)%
				IC_{50} (μM ± SD)[a]		IC_{50} COX-1/IC_{50} COX-2		
9	 **5i**	44.53	17.85	29.55 ± 0.19	71.25 ± 1.29	41%	62.53	57.85
10	 **5j**	21.70	7.89	ND	ND	ND	77.70	67.89
11	 **5k**	12.26	4.45	ND	ND	ND	87.26	74.45
12	 **5l**	47.74	19.78	31.25 ± 0.24	58.75 ± 1.58	36%	42.74	39.78
13	 **5m**	20.75	5.78	ND	ND	ND	79.54	75.78

(*continued on next page*)

Table 1 (*continued*)

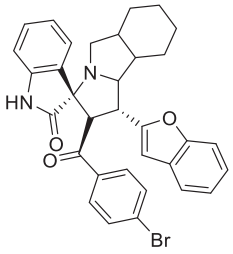
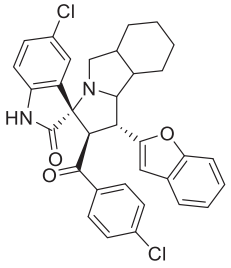
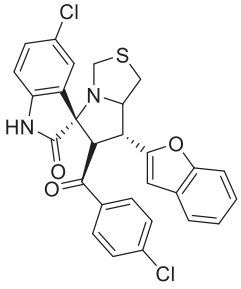
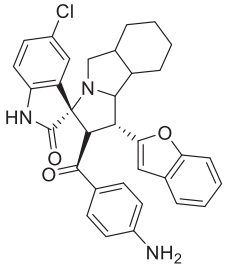
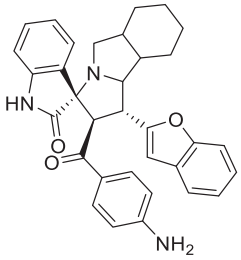
#	Compound	COX-1%	COX-2%	COX-1	COX-2	Selectivity[b]	IL-6 (pg/ml)%	TNF-α (pg/ml)%
				IC_{50} (μM ± SD)[a]		IC_{50} COX-1/IC_{50} COX-2		
14	 **5n**	9.43	1.11	ND	ND	ND	82.12	77.11
15	 **5o**	29.25	6.11	ND	ND	ND	76.81	66.11
16	 **5p**	52.75	9.98	38.24 ± 0.96	85.70 ± 1.51	45%	47.18	39.98
17	 **5q**	32.08	10.1	ND	ND	ND	67.43	60.1
18	 **5r**	32.08	6.91	ND	ND	ND	64.81	62.91

Table 1 (*continued*)

#	Compound	COX-1%	COX-2%	COX-1	COX-2	Selectivity[b]	IL-6 (pg/ml)%	TNF-α (pg/ml)%
				IC_{50} (μM ± SD)[a]		IC_{50} COX-1/IC_{50} COX-2		
STD	Indomethacin	87.31	18.76	0.24 ± 0.05	3.28 ± 0.09	7%	7.7	8.1
	Acetyl-keto boswellic acid	71.42	19.87	7.84 ± 0.13	82.52 ± 0.62	10%	7.31	18.76
	Acetyl-β-boswellic acid	75.73	14.87	8.16 ± 0.10	72.52 ± 0.51	11%	11.42	19.87
	Acetyl-α-boswellic acid	69.81	7.98	10.13 ± 0.17	>100	10%	15.73	14.87
	β-Boswellic acid	64.77	7.66	18.14 ± 0.42	>100	18%	19.81	27.98
	Celecoxib	8.7	92	29.19 ± 0.33	0.08 ± 0.44	365%	24.77	25.16
	Lipopolysaccharide (LPS)	–	–	–	–	–	100	100

[a] COX-1, and COX-2 inhibitory activity is expressed as the mean ± SD of triplicate experiments. [b]Selectivity is defined as IC_{50} COX-1/IC_{50} COX-2. [c]ND: not determined.

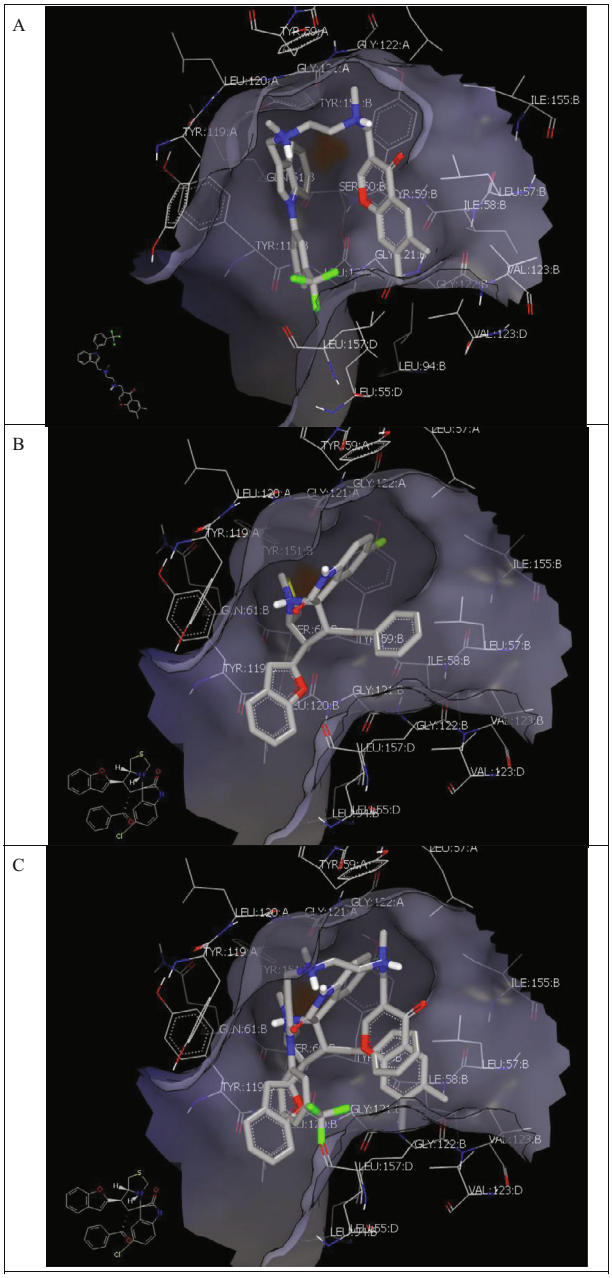


Fig. 3. **(A)** Standard drug co-crystallized with (ID: 2az5) through the formation of hydrophobic-hydrophobic interactions. (**B**) Compound **5a** with (ID: 2az5) docked outside the receptor through the formation of hydrophobic-hydrophobic interactions. **(C)** Compound **5a** with (ID: 2az5) docked with receptor overlay with standard drugs through the formation of hydrophobic-hydrophobic interactions.

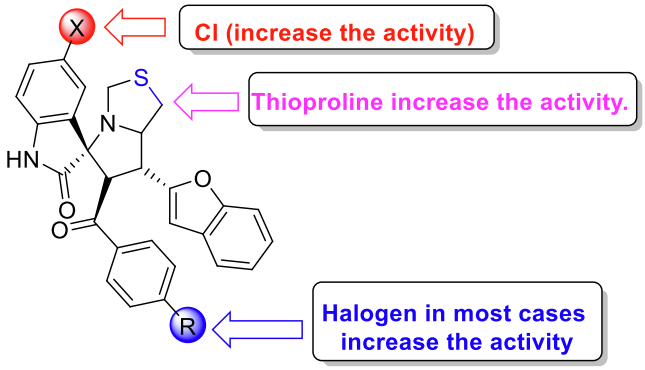


Fig. 4. SAR of the studied compounds **5a-r.**

and IC_{50} = 20.42 ± 0.55 μM against COX-1 compared to the standard drug, celecoxib (8.7% inhibition and IC_{50} = 29.19 ± 0.33 μM).

(iii) Compound **5c,** with a chlorine atom on the oxindole moiety, and *para* fluoro-substituted benzene on the benzoyl nucleus, showed good inhibition with 40.81% and IC_{50} = 37 ± 1.54 μM against COX-1, on the other hands when the chlorine and fluorine atoms replaced with bromine there is not dramatically changes in the activity which has been shown slightly more inhibition with 41.70% and IC_{50} = 35.70 ± 0.51 μM but when chlorine combined with bromine in the hit **5i** shown better activity (44.53% with IC_{50} = 29.55 ± 0.19 μM) than **5c** and **5 h.**

(iv) Hits **5 l** (*para*-CF_3), and **5p** (*para*-Cl) with the 5-chlorooxindole moiety, the percentage of inhibition increases and the IC_{50} decreases respectively.

(v) Compound **5i**, with a chlorine atom on the oxindole moiety and a bromo-substituted benzene on the benzoyl nucleus, showed the best results with 62.53% inhibition of IL-6 compared to the standard drug, celecoxib (7.7% inhibition).

(vi) The benzofuran core structure plays a crucial rule in binding to the receptor via the formation of hydrogen bonds with the oxygen atom.

4. Conclusions

The studied compounds **5a-r** were resynthesized and evaluated *in vitro* against COX-1, and COX-2 enzymes. The *in vitro* assay results revealed that compounds **5a, 5c, 5h, 5i, 5l**, and **5p** fixed with a substituted (aryl or oxoindole) moiety were the most potent and selective for COX-1 inhibitor with IC_{50} values ranging between 20.42 and 38.24 μM. Additionally, compounds **5d**, **5e**, **5f**, **5g**, and **5k** showed high potency against the release of pro-inflammatory

cytokines such as IL-6, and TNF-α. The docking study reinforced the importance of the benzofuran moiety in the binding interaction especially through the formation of HB with receptors. This study illustrated that these could be useful lead compounds in the development of more potent and selective anti-inflammatory agents. Further *in vivo* studies will be considered for better understanding.

Declaration of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by deanship of scientific research at Princess Nourah Bint Abdulrahman University (Grant No#: 39-S-254).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.02.010. The synthetic details of the studied compounds along with the biological activity assays and docking studies protocols are provided in the supplementary information.

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