Article

Pharmacological Evaluation and Molecular Docking of Novel Synthesized Pyrazolopyrimidine Derivatives

Rawan Bafail *, Waad Samman , Marwa Alsulaimany , Ahmed Aljohani

Posted Date: 8 October 2023

doi: 10.20944/preprints202310.0436.v1

Keywords: Pharmacological Evaluation; Docking; Anti-inflammatory; Analgesic; Antimicrobial

Article

Pharmacological Evaluation and Molecular Docking of Novel Synthesized Pyrazolopyrimidine Derivatives

Rawan S. M. Bafail [1,*], Waad A. Samman [2], Marwa Alsulaimany [3] and Ahmed K. B. Aljohani [3]

[1] Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, Taibah University, Madinah Munawara, Saudi Arabia
[2] Department of Pharmacology and Toxicology, College of Pharmacy, Taibah University, Madinah Munawara, Saudi Arabia
[3] Department of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University, Madinah Munawara, Saudi Arabia
* Correspondence: Correspondence: rbafail@taibahu.edu.sa; rawansaeed610@yahoo.com.

Abstract: A series of pyrazolopyrimidines derivatives **4– 11** were synthesized using 1-(6-(3-methoxyphenyl)-4-methylpyridazin-3-yl)-1H-pyrazolo [3,4-d] pyrimidin-4-amine **3** as a starting material. The detailed synthesis, structure assignments of the novel compounds based on chemical and spectroscopic evidence, spectroscopic data, pharmacological properties and molecular docking are reported. The pharmacological evaluation illustrated that many of these compounds have good analgesic, anti-inflammatory and antimicrobial activities.

Keywords: pharmacological evaluation; docking; anti-inflammatory; analgesic; antimicrobial

1. Introduction

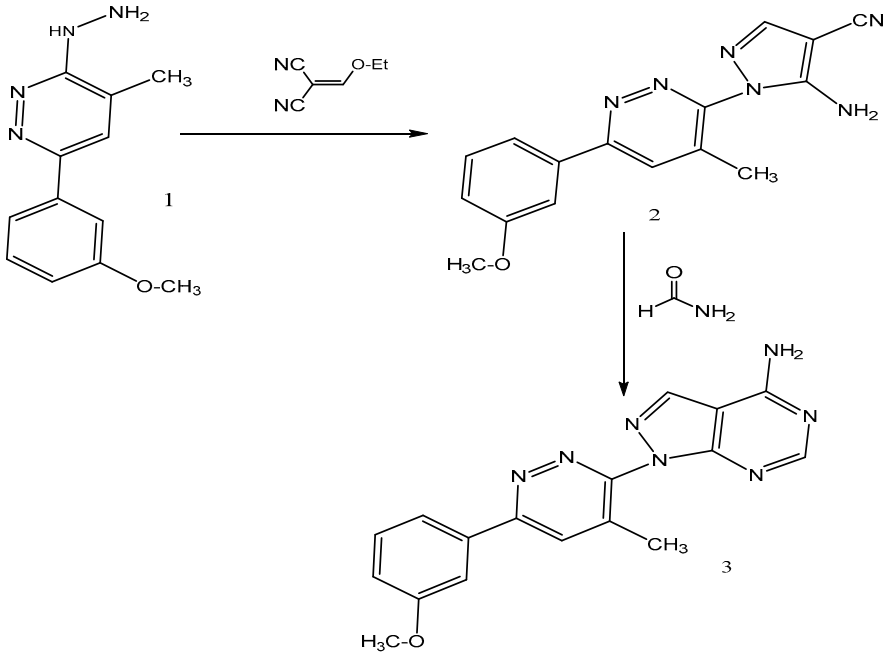
Heterocyclic nitrogen compounds are responsible for the mechanism of the drug and pharmacological activity [1]. Pyrimidine derivatives are a favored heterocyclic scaffolds that can act as ligands for various receptors in the body [2]. Pyrimidine ring can be found in nucleoside antibiotics so, they exhibit antimicrobial such as antibacterials [3]. Pyrimidine and its derivatives have been proven to be used as anti-neuroinflammatory, neuroprotective, analgesic agents [4–8]. They also, have activities against β-site amyloid precursor protein cleaving enzyme-1 (BACE-1), monoamine oxidases and cholinesterases [9]. Pyrimidines have been shown to be effective against *Pneumocystis carinii* (tg) and *Toxoplasma gondii* culture of tumor cell lines [10–12], so they had antitumor, anticancer activity as well as antiproliferative properties [13.14]. Pyrimidine derivatives have been previously reported to be α-adrenoceptor platelet-aggregation inhibitors, antagonists, anti-hypertensive, cardio-vascular, anti-parkinsonism and antinociceptive [15–18], as well as agro chemical and veterin products [19,20].

2. Results and Discussion

2.1. Chemistry

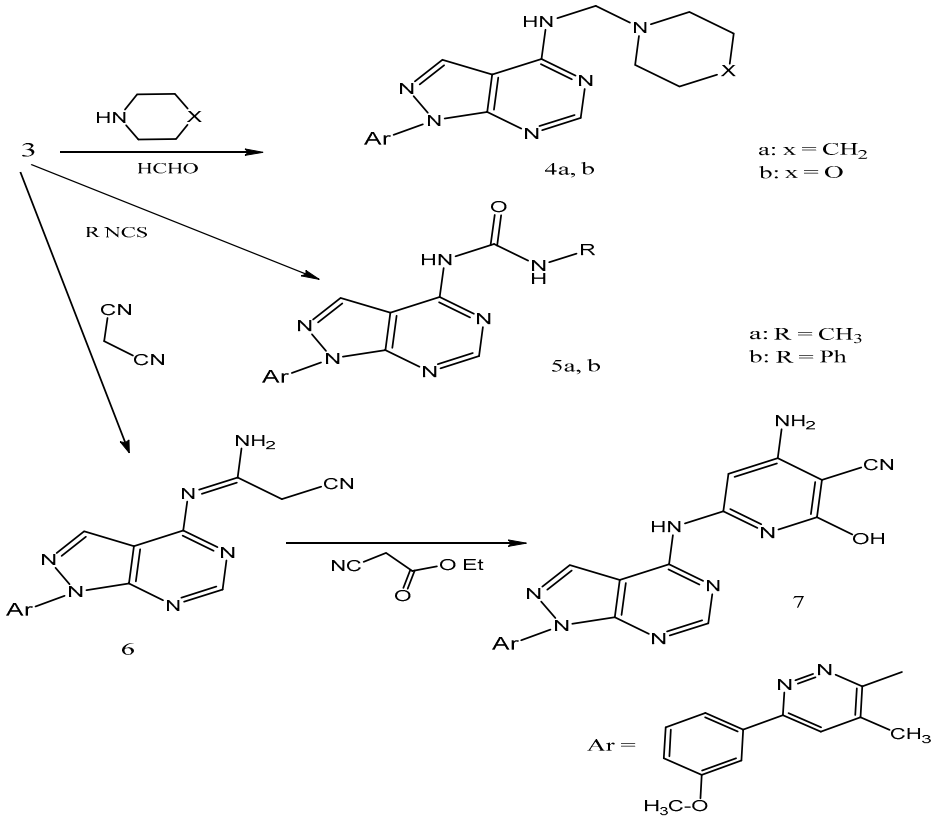
3-hydrazineyl-6-(3-methoxyphenyl)-4-methylpyridazine **1** used to synthesis a series of new pyrazolopyrimidine derivatives by reacting 1 with ethoxy methylene malononitrile gave 5-amino-1-(6-(3-methoxyphenyl)-4-methylpyridazin-3-yl)-4,5-dihydro-1H-pyrazole-4-carbonitrile **2** which, gave amino pyrazolopyrimidine 3 as starting material by reacted with formamide according to the reported procedures [21,22]. (Scheme 1)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 October 2023 doi:10.20944/preprints202310.0436.v1



Scheme 1. Synthetic route for compounds 1 - 3.

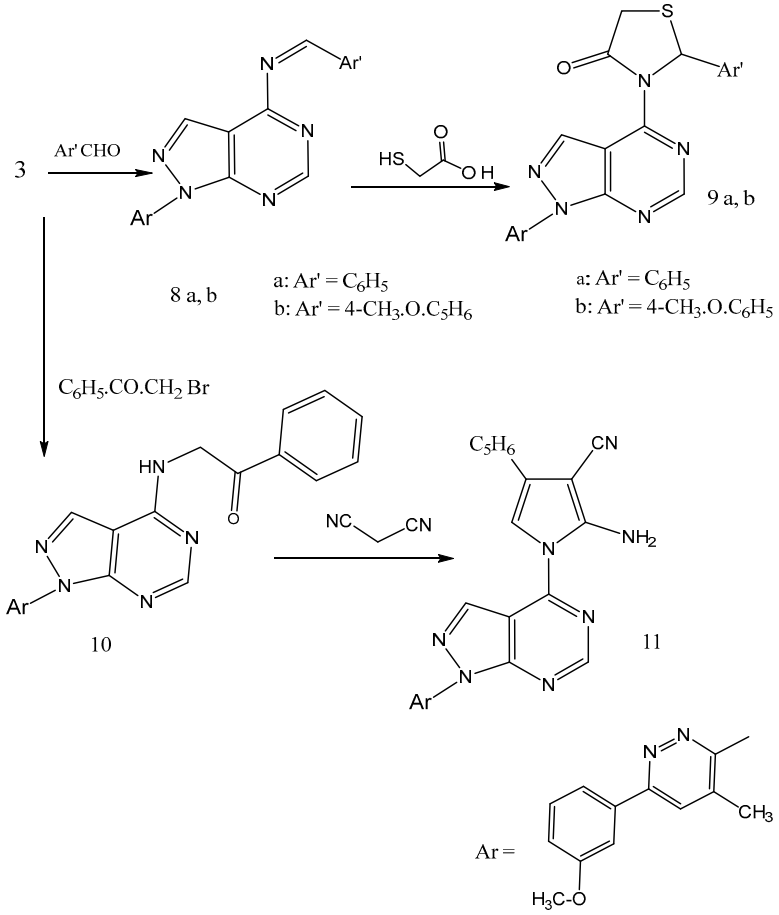
Reaction of compound 3 with piperidine or morpholine, and formaldehyde by Mannich reaction gave derivatives 4a, 4b. Compound 3 reacted with phenylisothiocyanate or methylisothiocyanate with formation of pyrazolopyrimidine derivatives 5a, 5b, respectively. Reaction of compound 3 with Malononitrile afforded intermediate 6, reaction of compound 6 with ethylcyanoacetate gave the corresponding pyrazolopyrimidine derivative 7. (Scheme 2)



Scheme 2. Synthetic route for compounds 4a,b - 7.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 October 2023 doi:10.20944/preprints202310.0436.v1

Also, compound 3 reacted with benzaldehyde or 4-methoxybenzaldehyde affording pyrazolopyrimidine derivatives 8a, 8b, respectively. Reaction of compounds 8a,8b with thioglycolic acid afforded compounds 9a, 9b. Phenacyl bromide reacted with compound 3 to give intermediate 10, which reacted with malononotrile to afford compound 11 (Scheme 3).



Scheme 3. Synthetic route for compounds $8_{a,b}$ - 11.

2.2. Pharmacological Activities

2.2.1. Anti-inflammatory Activity

Anti-inflammatory, ulcerogenicity and acute toxicity of Eight compounds **4a, 4b, 5a, 5b, 7, 9a, 9b** and **11** were studied. The result show that compounds **4a, 5b** and **9a** possessed weak anti-inflammatory activity from 7.3 ± 1.1 to 19.3 ± 1.2% of inhibition compared to Indomethacin (44.7 ± 1.2 - 61.2 ± 1.3%). Compounds **3, 4** and **9b** possessed moderate anti-inflammatory activity from 19.4 ± 1.6 to 26.5 ± 1.2% of inhibition compared to Indomethacin (49.2± 1.1 - 62.2± 1.2%). Compounds **7** and **11** possessed strong anti-inflammatory activity from 49.2± 1.1 to 62.2± 1.2 % of inhibition compared to Indomethacin (51.3 ± 1.2 - 63.5 ± 1.4%). (Figure 1).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 October 2023 doi:10.20944/preprints202310.0436.v1

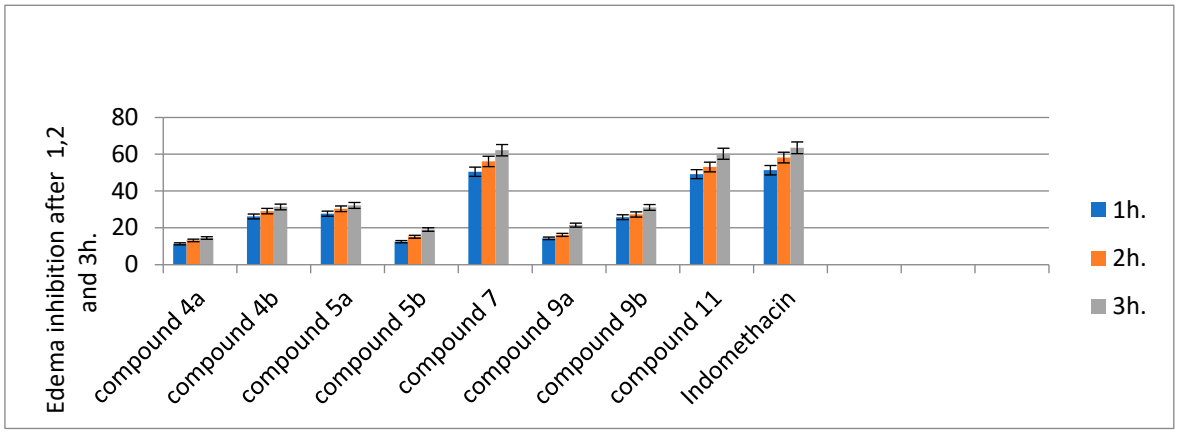


Figure 1. Anti-inflammatory activity of the synthesized compounds .

2.2.2. Ulcerogenicity

Screened of ulcerogenic activity of compound**s 7** and **11** showed no activity of 1.2 to 1.9 mm at dose levels of 10, 25 and 50 mg/kg b.m. (Table 1).

Table 1. Gastric ulceration in mice[a].

Compd. No.	Dose (mg/kg)[a]		
	10	25	50
Control	0/4	0/4	0/4
7	0/4(0)	0/4(0)	0/4(0)
11	0/4(0)	0/4(0)	0/4(0)
Indonethacin	1/4(1.2 ± 0.3)b.c	2/4(1.5 ± 0.23b.c	4/4(1.9 ± 0.3)b.c

a. number of mice lesions bigger than 0.5 mm in length per total no of mice. b. mean ulcer lesions ± SEM(mm) (n=4) in parentheses. c. significant difference at $p \leq 0.05$ compared to the control.

2.2.3. Acute toxicity

By injecting various gradually increasing doses of the tested compounds into adult male mice, the LD_{50} were determined for compounds **4b, 5a, 7, 9b** and **11**, then the dose corresponding to the death of the animal is calculated at 50% (Table 2).

Table 2. Acute toxicity (LD_{50}) of selected compounds.

Compound No.	**LD_{50}[mg/kg]**
4b	1.94 ± 0.01
5a	2.01 ± 0.02
7	1.25 ± 0.03
9b	2.23 ± 0.01
11	1.14 ± 0.01
Indomethacin	1.81 ± 0.02

2.3. Analgesic activity

All tested compounds showed analgesic activities in the hot plate test. Comparison of analgesic activity of test compounds to Valdecoxib® after 30 min showed that compounds **4a**, **5b** and **9a** possessed weak analgesic activities (0.18± 0.01, 0.19± 0.02, 0.22± 0.02) respectively. Compounds **4b**, **5a** and **9b** possessed intermediate activities (0.39± 0.03, 0.48± 0.03, 0.58± 0.01), respectively. Compound **11** possessed strong activities (0.81± 0.03), compound **7** possessed very strong activities (0.89± 0.01) in comparison to that of Valdecoxib® (1.00± 0.03). After 60 min compounds **4a**, **5b** and **9a** possessed weak analgesic activities (0.21± 0.02, 0.23± 0.02, 0.26± 0.05) respectively. Compounds **4b**, **5a** and **9b**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 October 2023 doi:10.20944/preprints202310.0436.v1

possessed intermediate activities (0.41± 0.04, 0.51± 0.03, 0.61± 0.01), respectively. Compound **11** possessed strong activities (0.86± 0.01), compound **7** possessed very strong activities (0.91± 0.03) in comparison to that of Valdecoxib® (1.00± 0.04). After 90 min showed that compounds **4a**, **5b** and **9a** possessed weak analgesic activities (0.27± 0.05, 0.29± 0.01, 0.31± 0.02) respectively. Compounds **4b**, **5a** and **9b** possessed intermediate activities (0.43± 0.03, 0.54± 0.02, 0.63± 0.05), respectively. Compound **11** possessed strong activities (0.86± 0.01), compound **7** possessed very strong activity (0.91± 0.02) compared to valdecoxib® (1.00± 0.02) (Figure 2).

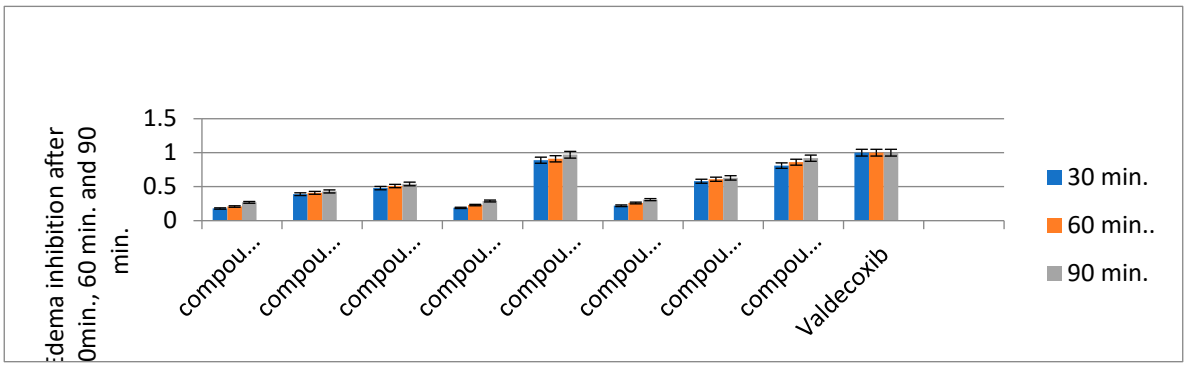


Figure 2. Analgesic activity of tested compounds.

2.4. Antimicrobial Activity

According to the modified Kirby-Bauer's disk diffusion method, antimicrobial activities of novel synthesized compounds at different applied concentration, (1000, 5000, 10000 ppm) against four bacterial strains *E. Coli, S. typhi, Bucillus subtilis* and *S. aureus* and *Aspergillus niger* as fungal strains and also two strain of yeast *Candida albicans* and *Sacchromyces* were determined. By the tube dilution technique MIC values of the tested compounds were determined.

"Streptomycin and Erythromycin were used as standards and the solvents DMSO/DMF were used as negative controls, Average diameters (for triplicate sets) of the zones of inhibition (in mm) for test samples were calculated compared with that produced by the standard drugs". Almost, all compounds tested were had to exhibit antimicrobial activities. Compounds **7** and **11** had higher significant against bacterial strains, fungal strains and yeast activities than standard drugs. However, compound **4b, 5a** and **9b** showed moderate inhibitions against bacterial strains. Compounds **4a**, **5b** and **9a** showed in general weak inhibitions against bacterial strains, fungal strains and yeast. (Table 3). The results are summarized in a Table 3 with the average diameter of the inhibition zone in mm

Table 3. Antimicrobial activities of the tested compounds.

Tested Comp. No.	Diameter of inhibition Zone (mm)[a] (MIC values are in µg/mL)						
	Microorganism						
	Bacteria Gram –ve		Bacteria Gram +ve		Fungi	Yeast	
	E. Coli	*S. typhi*	*B. subtitls*	*S. aureus*	*A. niger*	*C. Albicans*	*Sacchromyces*
Control (DMSO/DMF)	0	0	0	0	0	0	0
4a	6	4	7	10	9	11	13
4b	13	14	12	16	15	9	15
5a	14	13	14	17	16	11	17
5b	8	7	8	5	12	10	14
7	26	23	21	29	30	18	28
9a	10	8	11	7	13	11	15
9b	15	13	13	17	18	12	18

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 October 2023 doi:10.20944/preprints202310.0436.v1

11	25	21	20	28	29	16	27
Streptomycin [b]	28	26	25	30	32	20	31
Erythromycin [b]	10	8	21	17	19	6	9

[a]13mm or less: no inhibition or resistant, 14-18mm: moderate inhibition, 19mm or more: maximum inhibition
[b]The concentration 30 µg/mL.

2.5. The binding affinity of the synthesized 7 compound into FabH, and prostaglandin H2 synthase receptor.

"Each of two co -crystalized ligand (MLC), (IMM), and compound 7 were docked into active site of FabH and for prostaglandin H2 synthase receptor, respectively. The binding affinities were evaluated on the basis of the binding free energy S-score and hydrogen bonds with their distance between the designed compounds and the amino acids in the receptor". (Table 4 & Figure 3 & Figure 4).

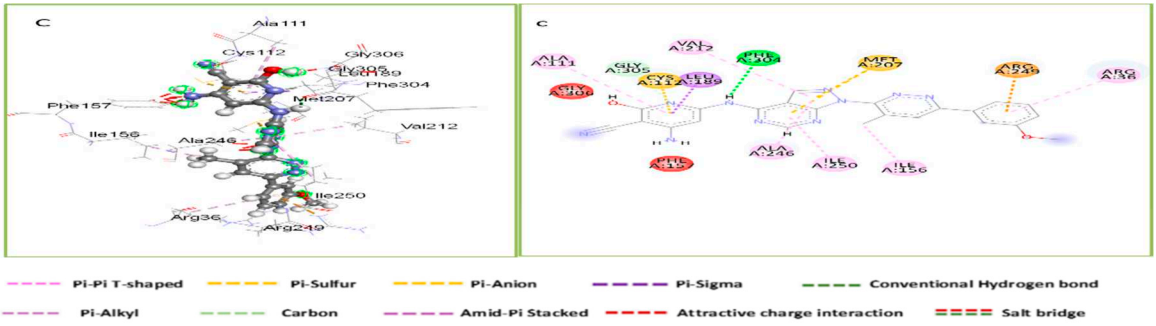


Figure 3. Docking mode of compound 7 into FabH receptor: (C) binding sites & (c) model).

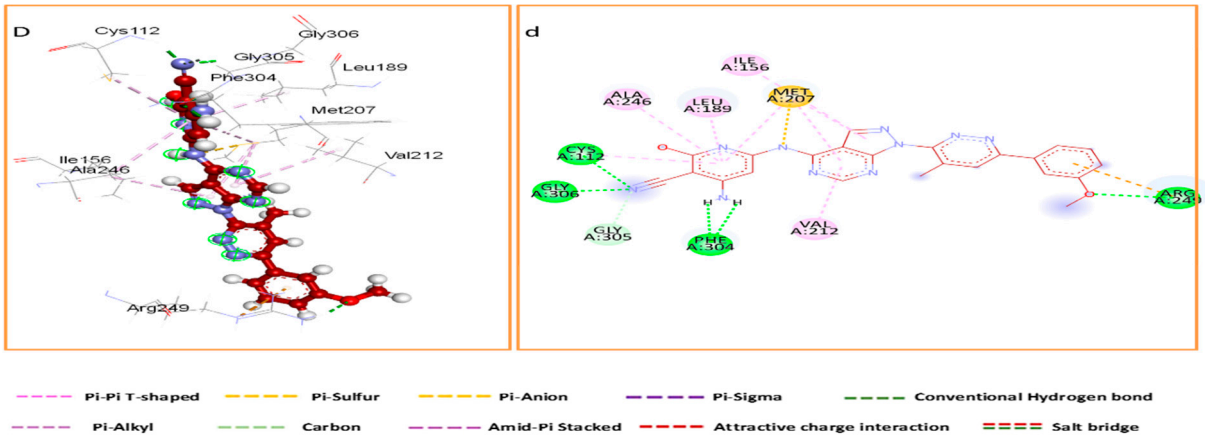


Figure 4. Docking mode of compound 7 into Prostaglandin H2 Synthase receptor receptor: (D) binding sites & (d) model).

Table 4. Summary of Molecular Operating Environment (MOE) docking.

Ligands	*Hydrogen bonds between atoms of ligands and amino acids of receptor*					**S- score** (binding energy) (kcal/mol)
	ligands Atoms	Receptor Atoms	Residues	Type	Distance (Å)	
FabH Receptor						
MLC	H 4701	O 2225	Arg 151	H-donor	2.15	-13.96
	N 4697	OG 439	Thr 28	H-acceptor	2.99	
	O 4706	NH 2241	Arg 151	H-acceptor	2.93	

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 October 2023 doi:10.20944/preprints202310.0436.v1

	O	4743	ND 3645	Asn	247	H-acceptor	3.09	
7	H	4736	O 4486	Phe	304	H-donor	2.01	-14.38
	N	4722	N 1659	Cys	112	H-acceptor	2.83	
	N	4698	ND 3645	Asn	247	H-acceptor	2.52	
	N	4722	N 4508	Gly	306	H-acceptor		
Prostaglandin H2 Synthase Receptor								
IMM	O	8914	NH 1437	ARG	120	H-donor	2.67	-12.53
7	O	8906	OH 5754	TYR	385	H-donor	2.97	-13.34
	H	8926	O 7926	MET	522	H-donor	2.35	
	O	8906	OG 8049	Ser 530		H-donor	2.37	
	N	8887	NH 1437	ARG	120	H-acceptor	1.05	

3. Materials and methods

3.1. Chemistry

"Melting points were determined on open glass capillaries using an Electrothermal IA 9000 SERIES digital melting point apparatus (Electrothermal, Essex, U.K.) and are uncorrected. The IR spectra (KBr) were recorded on a FT IR-8201 PC spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR were measured with Jeol FTGNM-EX 270, 270 MHz instrument in DMSO-d_6 and the chemical shifts were recorded in (δ, ppm) relative to TMS. The Mass spectra were run at 70 eV with a Finnigan SSQ 7000 spectrometer using EI and the values of m/z are indicated in Dalton. TLC (Silica gel, aluminum sheets 60F_{254}, Merck, Darmstadt, Germany) followed the reactions".

Synthesis of 5-amino-1- (6-(3-methoxyphenyl) -4-methylpyridazin-3-yl) -1H- pyrazole -4-carbonitrile **(2)**. A mixture of compound 1 (1 mmol) and ethoxymethylene malononitrile (1 mmol) was refluxed in ethanol (30 mL) for 3 h. Evaporated the solvent under reduced pressure; Crystallized the solid product from ethanol to afford compound 2 as a yellow powder. Yield: 79%; m.p.: 257–258 °C; IR spectrum, ν, cm^{-1}: 3341–3310 (NH_2) and 2212 (CN) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 2.31 (s, 3H, CH_3), 3.231 (s, 3H, CH_3), 6.92–7.85 (m, 6H, 4Ar–H, 1H$_{pyridazine}$ and 1H$_{Pyrazolo}$) and 9.62 (b, 2H, NH_2, D_2O -exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 24.7(CH_3), 45.2(OCH_3), 114.6, 1119.4, 128.1, 129.5, 131.1, 145.5 (Ar–C), 119.4 (CN), 124.6, 131.2, 140.8, 151.9 (pyridazin$_e$–C), 135.3, 138.1, 141.6 (pyrazole–C) ppm; ms: m/z 306 (M+, 60) and 198 (100, base peak); Anal. Calcd for $C_{16}H_{14}N_6O$ (306.32): C, 62.72; H, 4.59; N, 27.42. Found: C, 62.41; H, 4.37; N, 27.14.

Synthesis of 1-(6-(3-methoxyphenyl)-4-methylpyridazin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine **(3).** A solution of compound 3 (1 mmol) was refluxed in formamide (25 mL) for 3 h; after cooling, collected the product by filtration, and crystallized from methanol to afford 0.22 g of compound 3 as a reddish-brown powder. Yield 69%, mp 298-299°C; IR spectrum, ν, cm^{-1}: 3342 (NH2) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.84 (s, 3H, CH_3), 3.18 (s, 3H, OCH_3), 7.11–7.41 (m, 7H, Ar-H), 10.84 (br,s, 2H, NH_2, D_2O -exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 19.36 (CH3), 23.17 (OCH3), 138.25, 139.47, 140.16, 141.21 (pyridazine-C), 136.15, 137.21, 138.91, 140.74, 142.18 (pyrazolopyrimidine-C), 112.53, 117.64, 125.48, 129.23, 137.54, 149.27 (Ar-C) ppm; ms: m/z 333 (M+ ,29) and 135 (100, base peak); Anal. Calcd for $C_{17}H_{15}N_7O$ (333.35): C, 61.24; H, 4.51; N, 29.40. Found: C, 61.02; H, 4.26; N, 29.21.

Synthesis of compounds (**4a, 4b).** A mixture of compound 3 (1 mmol) with either morpholine or piperidine (1 mmol) and formaldehyde (2 mmol) was refluxed in ethanol for 4 h. Poured the reaction mixture onto crushed ice onto crushed ice. Recrystallized the product from the appropriate solvent to afford the corresponding compound 4a or 4b.

1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl)-N- (piperidin-1-ylmethyl) -1H- pyrazolo [3,4-d] pyrimidin -4- amine **(4a).** Yield 63%, mp 232–234°C. (EtOH). IR spectrum, ν, cm^{-1}: 3338(NH), 1552 (C=C), 1381 (C=N); ^{1}H-NMR (DMSO-d_6): δ = 1.28–2.64 (m, 10H, 5CH2), 2.83 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 4.15 (s, 2H, CH_2), 6.13 (s, 1H, NH, D_2O -exchangeable), 7.17–7.69 (m, 7H, 4Ar–H, 1H pyridazine, 1H pyrazole, 1H pyrimidine) ppm; ^{13}C-NMR (DMSO-d_6): δ = 17.5 (CH_3), 21.4, 23.9, 39.1, 43.7, 46.8, 56.4 (6CH_2), 54.7 (OCH_3), 113.5, 118.4, 123.1, 132.7, 141.2, 132.5 (Ar-C), 123.1, 126.8, 147.3,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 October 2023 doi:10.20944/preprints202310.0436.v1

151.4 (pyridazine–C), 124.5, 131.3, 139.8 (pyrazole–C), 151.4, 154.6 (pyrimidine–C), ppm; ms: m/z 430 (M+ , 38) and 179 (100, base peak); Anal. Calcd for $C_{23}H_{26}N_8O$ (430.51): C, 64.17; H, 6.09; N, 26.03; O, 3.72. Found: C, 64.01; H, 5.91; N, 25.89; O, 3.56.

1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -N- (morpholinomethyl) -1H- pyrazolo [3,4-d] pyrimidin -4-amine **(4b).** Yield 69%, mp 261–263°C. (EtOH). IR spectrum, ν, cm^{-1}: 3341 (NH), 1546 (C=C), 1385 (C=N), 1371 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ = 1.35–2.74 (m, 8H, $4CH_2$), 2.76 (s, 3H, CH_3), 3.52 (s, 3H, OCH_3), 4.23 (s, 2H, CH_2), 6.43 (s, 1H, NH, D_2O -exchangeable), 6.87–7.38 (m, 7H, 4Ar–H, 1H pyridazine, 1H pyrazole, 1H pyrimidine) ppm; ^{13}C-NMR (DMSO-d_6): δ = 16.4 (CH_3), 22.3, 25.6, 41.2, 46.4, 53.1 ($5CH_2$), 53.8 (OCH_3), 116.2, 119.5, 124.3, 133.4, 140.8, 133.2 (Ar-C), 124.5, 127.3, 146.8, 152.6 (pyridazine–C), 123.7, 132.4, 138.6 (pyrazole–C), 152.3, 156.8 (pyrimidine–C), ppm; ms: m/z 432 (M+,42) and 221 (100, base peak); Anal. Calcd for $C_{22}H_{24}N_8O_2$ (432.47): C, 61.09; H, 5.58; N, 25.90; O, 7.40, Found: C, 59.94; H, 5.41; N, 25.74; O, 7.23.

Synthesis of compounds **(5a,5b).** A mixture of compound 3 (1 mmol) with phenylisothiocyanate or methylisothiocyanate (3 mmol) in dimethyl formamide (20 mL) and few drops of triethyl amine was heated at 90°C (water bath) for 8 h. The solid product was filtered off, washed with water and crystallized from the proper solvent to afford the corresponding compounds 5a, 5b.

1-(1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H- pyrazolo [3,4-d] pyrimidin-4-yl) -3-phenylurea **(5a).** Yield 72%, mp 228–230°C. (MeOH). IR spectrum, ν, cm^{-1}: 3365, 3348 (2NH), 1660 (C=O). ^{1}H-NMR (DMSO-d_6): δ = 2.38 (s, 3H, CH_3), 3.16 (s, 3H, OCH_3), 6.94–7.42 (m, 12H, 9Ar–H, 1H pyridazine, 1H pyrazole, 1H pyrimidine), 9.72, 10.21 2s (2H, 2 NH, D_2O -exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 18.5 (CH_3), 43.6 (O CH_3), 121.7, 123.5, 126.8, 129.3, 130.1, 132.2, 133.8, 135.4, 136.3, 137.1, 140.2, 143.8 (Ar-C), 126.5, 147.5, 149.8, 150.6 (pyridazine–C), 125.2, 131.7, 139.4 (pyrazole–C), 151.7, 157.4 (pyrimidine–C), 153.18 (C = O) ppm; m/z 452 (M+,29) and 178 (100, base peak); Anal. Calcd for $C_{24}H_{20}N_8O_2$ (452.48): C, 63.70; H, 4.46; N, 24.76; O, 7.07, Found: C, 63.59; H, 4.27; N, 24.53; O, 6.94.

1-(1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H- pyrazolo [3,4-d] pyrimidin-4-yl) -3-methylurea **(5b).** Yield 65%, mp 274–276°C. (EtOH). IR spectrum, ν, cm^{-1}: 3346, 3329 (2NH), 1659 (C=O). ^{1}H-NMR (DMSO-d_6): δ = 2.62 (s, 3H, CH3), 3.21 (s, 3H, CH_3), 4.82 (s, 3H, O CH_3), 7.12–7.53 (m, 7H, 4Ar–H, 1H pyridazine, 1H pyrazole, 1H pyrimidine), 8.97, 10.31 (2s, 2H, 2 NH, D_2O -exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 21.4 (CH_3), 35.2 (CH_3), 47.3 (O CH_3), 122.4, 127.1, 130.6, 133.3, 136.6, 142.5 (Ar-C), 131.8, 148.4, 150.3, 151.1 (pyridazine–C), 128.2, 132.5, 138.7 (pyrazole–C), 152.4, 156.5 (pyrimidine–C), 155.6 (C = O) ppm; m/z 390 (M+,56) and 254 (100, base peak); Anal. Calcd for $C_{19}H_{18}N_8O_1$ (390.41): C, 58.45; H, 4.65; N, 28.70; O, 8.20, Found: C, 58.28; H, 4.47; N, 28.53; O, 7.97.

2-cyano -N- (1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H- pyrazolo [3,4-d] pyrimidin-4-yl) acetimidamide **(6).** A mixture of compound 3 (2 mmol) in sodium ethoxide (2 mmol, 50 mL) and malononitrile was added (2 mmol). The reaction mixture was refluxed for 4 h, then poured onto ice–water mixture containing a few drops of conc. HCl. The solid product recrystallized from ethanol. Yield 68%, mp 294-296°C. IR spectrum, ν, cm^{-1}: 3335(NH_2), 2224 (CN). ^{1}H-NMR (DMSO-d_6): δ = 2.32 (s, 3H, CH_3), 3.47 (s, 3H, O CH_3), 3.65 s (2H, CH_2), 6.74–7.13 (m, 7H, 4Ar–H, 1H pyridazine, 1H pyrazole, 1H pyrimidine), 10.23 s (2H, NH2, D_2O -exchangeable). ppm; m/z 399 (M+,71) and 201 (100, base peak); Anal. Calcd for $C_{20}H_{17}N_9O$ (399.42): C, 60.14; H, 4.29; N, 31.56; O, 4.01, Found: C, 60.01; H, 4.07; N, 31.74; O, 3.87.

Synthesis of 4-amino -2- hydroxyl -6- ((1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H-pyrazolo [3,4-d] pyrimidin-4-yl)amino) nicotinonitrile **(7).** To a suspended mixture of compound 6 (2 mmol) with sodium ethoxide (2 mmol, 50 mL) was added ethylcyanoacetate (2 mmol), and refluxed for 4 h then poured onto an ice–water mixture containing a few drops of conc. HCl. The solid product was collected and crystallized from ethanol to afford compound 7. Yield 58 %, mp 280–282°C. (EtOH). IR spectrum, ν, cm^{-1}: 3384-3269 ($2NH_2$, NH), 2221 (CN). ^{1}H-NMR (DMSO-d_6): δ = 2.31 (s, 3H, CH_3), 3.61(s, 3H, O CH_3), 6.74–7.13 (m, 8H, 4Ar–H, 1H pyridine, 1H pyridazine, 1H pyrazole, 1H pyrimidine), 8.98(s, 1H, NH, D_2O -exchangeable), 10.14 (s, 2H, NH_2, D2O-exchangeable),12.16 (s, 1H, OH, D_2O-exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 23.5 (CH_3), 42.1 (O CH_3), 117.6 (CN), 121.5, 128.3, 131.8, 134.7, 137.3, 143.8 (Ar-C), 131.8, 148.4, 150.3, 151.1 (pyridazine–C), 128.2, 132.5, 138.7

 doi:10.20944/preprints202310.0436.v1

(pyrazole–C), 152.4, 156.5 (pyrimidine–C), 127.8, 131.2, 139.4, 153.9, 158.3 (pyridine–C) ppm; m/z 466 (M+,29) and 255 (100, base peak); Anal. Calcd for $C_{23}H_{18}N_{10}O_2$ (466.47): C, 59.22; H, 3.89; N, 30.03; O, 6.86, Found: C, 59.01; H, 3.68; N, 29.94; O, 6.69.

Synthesis of compounds **(8a,8b).** A mixture of compound 3 (5 mmol) with benzaldehyde or p-methoxybenzaldehyde (5 mmol) in ethanol (30 mL) and few drops of piperidine was refluxed for 6 h. Concentrated the mixture, and filtered off, dried and the solid product recrystallized to afford compounds 8a, 8b.

N-(1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H- pyrazolo [3,4-d] pyrimidin-4-yl) -1-phenylmethanimine **(8a).** Yield 63 %, mp 256–258°C. (MeOH). IR spectrum, ν, cm^{-1}: 3054 (CH aromatic), 2938 (CH aliphatic), 1625 (C=N). ^{1}H-NMR (DMSO-d_6): δ = 2.52 (s, 3H, CH_3), 3.48 (s, 3H, O CH_3), 6.83–7.24 (m, 12H, 9Ar–H, 1H pyridazine, 1H pyrazole, 1H pyrimidine), 8.16 (s, 1H, N=CH) ppm; m/z 421 (M+,23) and 223 (100, base peak); Anal. Calcd for $C_{24}H_{19}N_7O$ (421.46): C, 68.40; H, 4.54; N, 23.26; O, 3.80, Found: C, 68.19; H, 4.38; N, 23.07; O, 3.59.

1-(4-methoxyphenyl) -N- (1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H- pyrazolo [3,4-d] pyrimidin-4-yl) methanimine **(8b).** Yield 71 %, mp 283–285°C. (MeOH). IR spectrum, ν, cm^{-1}: 3112 (CH aromatic), 2965 (CH aliphatic), 1622 (C=N). ^{1}H-NMR (DMSO-d_6): δ = 2.28 (s, 3H, CH_3), 3.16 (s, 3H, CH_3), 3.31 (s, 3H, O CH_3), 7.06–7.67 (m, 11H, 8Ar–H, 1H pyridazine, 1H pyrazole, 1H pyrimidine), 8.42 (s, 1H, N=CH) ppm; m/z 451 (M+,49) and 161 (100, base peak); Anal. Calcd for $C_{25}H_{21}N_7O_2$ (451.49): C, 66.51; H, 4.69; N, 21.72; O, 7.09, Found: C, 66.37; H, 4.48; N, 21.53; O, 6.98.

Synthesis of compounds **(9a,9b).** A Schiff base 8a or 8b (1 mmol) mixed with dry benzene (30 mL) then add thioglycolic acid (1 mmol) slowly. Refluxed the mixture for 6h. Evaporated the solvent and added bicarbonate solution. The product was recrystallized to give compound 9a or 9b.

3-(1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H- pyrazolo [3,4-d] pyrimidin-4-yl) -2-phenylthiazolidin -4- one **(9a).** Yield 68 %, mp 271–273°C. (EtOH). IR spectrum, ν, cm^{-1}: 1660 (C=O amide). ^{1}H-NMR (DMSO-d_6): δ = 2.28 (s, 3H, CH_3), 3.27 (s, 2H, CH_2 thiazole ring), 3.54 (s, 3H, O CH_3), 5.18 (s, 1H, N–CH–S), 6.92–7.46 (m, 12H, 9Ar–H, 1H pyridine, 1H pyridazine, 1H pyrazole, 1H pyrimidine), ppm; ^{13}C-NMR (DMSO-d_6): δ = 20.9 (CH_3), 33.1 (CH_2), 48.3 (O CH_3), 63.4 (CH), 119.4, 121.7, 123.2, 125.3, 128.3, 129.8, 133.4, 135.5, 138.2, 140.4, 143.8, 146.2 (Ar-C), 130.2, 147.6, 151.5, 153.3 (pyridazine–C), 129.4, 133.2, 139.4 (pyrazole–C), 153.4, 154.8 (pyrimidine–C), 168.2 (C=O) ppm; m/z 495 (M+,64) and 297 (100, base peak); Anal. Calcd for $C_{26}H_{21}N_7O_2S$ (495.56): C, 63.02; H, 4.27; N, 19.79; O, 6.46, Found: C, 62.88; H, 4.03; N, 19.54; O, 6.28.

2-(4-methoxyphenyl) -3- (1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H- pyrazolo [3,4-d] pyrimidin-4-yl) thiazolidin-4-one **(9b).** Yield 65 %, mp 294–296°C. (MeOH). IR spectrum, ν, cm^{-1}: 1662 (C=O amide). ^{1}H-NMR (DMSO-d_6): δ = 2.21 (s, 3H, CH_3), 3.19 (s, 2H, CH_2 thiazole ring), 3.36 (s, 3H, O CH_3), 3.41 (s, 3H, O CH_3), 5.23 (s, 1H, CH thiazole ring), 7.14–7.53 (m, 11H, 8Ar–H, 1H pyridine, 1H pyridazine, 1H pyrazole, 1H pyrimidine), ppm; ^{13}C-NMR (DMSO-d_6): δ = 20.3 (CH_3), 31.5 (CH_2), 46.5 (O CH_3), 51.1 (O CH_3), 71.6 (CH), 117.2, 122.3, 123.6, 126.7, 129.2, 131.4, 134.2, 138.8, 141.4, 143.4, 146.2, 148.2 (Ar-C), 131.7, 148.2, 152.8, 154.1 (pyridazine–C), 128.2, 137.8, 138.5 (pyrazole–C), 152.3, 156.5 (pyrimidine–C), 172.3 (C=O) ppm; m/z 525 (M+,51) and 209 (100, base peak); Anal. Calcd for $C_{27}H_{23}N_7O_3S$ (525.59): C, 61.69; H, 4.41; N, 18.66; O, 9.14, Found: C, 61.59; H, 4.24; N, 18.47; O, 9.01.

2-((1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H- pyrazolo [3,4-d] pyrimidin-4-yl)amino) -1-phenylethan-1-one **(10).** A mixture of compound 3 (1 mmol) and phenacyl bromide (1 mmol) in ethanol (30mL) was refluxed for 5 h. Filtered off and crystallized the product from dioxane to afford compound 10. Yield 66%, mp 288–290°C. (MeOH). IR spectrum, ν, cm^{-1}: 3331 (NH), 1690 (C=O ketone). ^{1}H-NMR (DMSO-d_6): δ = 2.37 (s, 3H, CH_3), 3.31 (s, 3H, O CH_3), 4.16 (s, 2H, CH_2), 6.82–7.37 (m, 12H, 9Ar–H, 1H pyridine, 1H pyridazine, 1H pyrazole, 1H pyrimidine), 9.19 (s, 1H, NH, D_2O -exchangeable)ppm; ^{13}C-NMR (DMSO-d_6): δ = 23.5 (CH_3), 51.2 (O CH_3), 62.5 (CH_2), 121.4, 121.4, 123.6, 126.4, 128.9, 129.3, 134.7, 135.2, 139.5, 144.1, 146.2, 148.4 (Ar-C), 131.3, 147.4, 150.8, 154.1 (pyridazine–C), 128.2, 137.5, 138.7 (pyrazole–C), 152.8, 153.6 (pyrimidine–C), 174.3 (C=O) ppm; m/z 451 (M+,29) and 253 (100, base peak); Anal. Calcd for $C_{25}H_{21}N_7O_2$ (451.49): C, 66.52; H, 4.69; N, 21.72; O, 7.09, Found: C, 66.32; H, 4.49; N, 21.43; O, 5.96.

2-amino -1- (1-(6-(3-methoxyphenyl) -4- methylpyridazin-3-yl) -1H-pyrazolo [3,4-d] pyrimidin-4-yl) -4-phenyl-1H-pyrrole-3-carbonitrile **(11).** A mixture of compound 10 (1 mmol) with malononotrile (1 mmol) in ethanol (20 mL) and sodium ethoxide (0.5 g) was refluxed for 4 h, after cooling, acidified with dil. HCl. Recrystallized the product from dioxane to afford compound 11. Yield 62%, mp over 300°C. (pet. ether). IR spectrum, ν, cm^{-1}: 3431 (NH_2), 2221 (CN). ^{1}H-NMR (DMSO-d_6): δ = 2.45 (s, 3H, CH_3), 3.86 (s, 3H, O CH_3), 7.06–7.51 (m, 13H, 9Ar–H, 1H pyrrole, 1H pyridazine, 1H pyrazole, 1H pyrimidine), 10.12 (s, 2H, NH_2, D_2O -exchangeable) ppm; ^{13}C-NMR (DMSO-d_6): δ = 24.5 (CH_3), 52.3 (O CH_3), 124.3 (CN), 119.2, 122.7, 124.5, 127.2, 128.2, 129.7, 133.4, 135.6, 138.4, 143.3, 145.2, 149.1 (Ar-C), 130.2, 135.9, 144.8, 152.6 (pyrrole–C), 132.2, 146.4, 151.5, 153.4 (pyridazine–C), 129.4, 137.8, 138.1 (pyrazole–C), 153.4, 155.2 (pyrimidine–C) ppm; m/z 499 (M+,51) and 214 (100, base peak); Anal. Calcd for $C_{28}H_{21}N_9O$ (499.54): C, 67.32; H, 4.24; N, 25.24; O, 3.20, Found: C, 67.13; H, 4.07; N, 25.06; O, 3.02.

3.2. Pharmacological screening

3.2.1. Animals

"Female albino mice 16 - 18 g and Sprague Dawley mice 100 g were obtained from the animal house of Taibah University (Madinah Munawara, KSA) in collaboration with King Saudi University (Riyadh, KSA). Experiments were approved by the Taibah University Animal Ethics Committee. Animals were maintained in accordance with accepted standards of animal care".

3.2.2. Anti-inflammatory Activity

"Newly synthesized derivatives were dissolved in 0.5% carboxymethyl cellulose (CMC) as a homogenate solution and administered intraperitonneally (IP). One hundred and eight rats were divided into eighteen groups, each group consisting of six animals. The anti-inflammatory activity of the compounds was studied in mice using carrageenan. A suspension of the tested compound and reference drug, indomethacin® in an aqueous solution was administered orally at a dose of 5 mg/kg. Control animals were treated with 0.5% CMC only. After 30 minutes, 0.1 mL of freshly prepared 1.0% carrageenan solution (formol saline) was injected into the sub-plantar region of the right paw [23]. Right paw volume was measured using a digital plethysmometer (Model 7150), directly before and after 1, 2, and 3 hrs., intervals after administration of the tested compounds".

3.2.2.1. Ulcerogenic activity

"Seventy-two mice were divided into twelve groups. Ulcerative activity was evaluated after oral administration of the tested compounds or indomethacin at doses of 10, 50, and 100 mg/kg. Control mice received 0.5% CMC. Food but not water was removed 24 hrs., before administration of the tested compounds. After 6 hrs., the mice were sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and gently cleaned by dipping into saline. Mucosal damage of each stomach was examined using a stereoscopic microscope and compared with the reference drug indomethacin according to the reported procedure" [24].

3.2.2.2. Acute Toxicity

"The median lethal doses (LD_{50}) of the most active compounds **4b, 5a, 7, 9b** and **11** were determined in mice [25]. Groups of male adult mice, each of six animals, were injected i.p. with graded doses of each of the test compounds. The percentage of mortality in each group of animals was determined 24 hrs., after injection. Computation of LD_{50} was processed by a graphical method".

3.3. Analgesic activity

"Sixty mice of both sexes were divided into ten groups. One group served as a control (received formalin saline), the second as a vehicle (acacia gum), and the third as a reference drug (Valdecoxib®), while the other groups received the test compounds subcutaneously (S.C.). Mice were gently

 doi:10.20944/preprints202310.0436.v1

deposited into a 1 Liter dry glass beaker kept at 55 to 55.5 °C. Normal reaction times for all animals were measured in seconds at intervals of 30, 60, and 90 minutes. This is the time elapsed between the mouse reaching the hot beaker and the animal licking its feet or jumping out of the beaker (dosage 5mg/kg) [26]. The efficacy of Valdecoxib were also determined".

3.4. Antimicrobial Screening

"Each of the tested compounds (0.5 g) was dissolved in 5 mL of dimethylformamide. A quantity of 0.1 mL of the test solution was placed on whatman paper disc of 9 mm diameter and the solvent was allowed to evaporate. These impregnated discs were carefully placed on the surface of the inoculated solid medium; each petri dish contains at least 3 discs. Petri dishes were incubated at 5 °C for an hour to allow good diffusion and then transferred to an 85 °C incubator overnight and then the results were examined and recorded by measuring the diameters of the inhibition zone" [27].

3.4.1. Microorganisms species

Bacteria:

a) Gram-negative bacteria, Escherichia coli, Salmonella typhi.
b) Gram-positive bacteria, Bacillus subtilis, Staphylococcus aureus.
1. Fungi: Aspergillus Niger.
2. Yeast: Candida albicans, Sacchromyces.

3.4.2. Medium

"The cap assay method was used containing (g/L): peptone 6.0, yeast extract 3.0, meat extract 1.5, glucose 1 and agar 20 were used. The medium was sterilized and divided while hot (50–60 °C) in 15 mL. Portions between sterile 9 cm diameter Petri dishes. One ml of Spore suspension of each microorganism was spread all over the surface of cold solid medium placed in a petri dish".

3.5. Molecular docking study

"Based on the in vitro pharmacological results, we selected compound 7 the best antibacterial, and anti-inflammatory inhibitor in this study, as the docking model (PDB ID: 1HNJ, and 1PGF), respectively [28,29]. Computer-guided docking experiments were carried out using Molecular Operating Environment (MOE 2015.10) software, Chemical Computing Group, Montreal, Canada. Molecular docking studies was studied to get deeper insight into the molecular bases of the inhibitory potency and for the purpose of lead optimization and to pick up the interaction between compounds and Ryanodine receptor".

3.5.1. Method

"The active ligands were prepared by hydrogens addition, partial charges calculation, and energy minimization using Force Field MMFF94x. Besides, the preparation of proteins was performed by omitting the repeating chains, water molecules, and surfactants. MOE Quick Prep functionality was used for correcting structural issues, 3D protonation, and calculation of partial charges. The default procedure in the MOE Dock protocol was utilized to detect the good binding poses of the studied ligands, using triangle matcher as placement method and London dG as the primary scoring function. An extra refinement step was set to rigid receptor method with GBVI/WSA dG scoring function to retain poses with the highest hydrophobic, ionic, and hydrogen-bond interactions with the protein. The output database comprised the scores of ligand enzyme complexes in kcal/mol. Then, the resulting docking poses were visually examined with BIOVIA Discovery Studio, and interactions with binding pocket residues were studied. Poses fitting into the binding pocket with the top scores and showing useful ligand enzyme contacts were selected".

3.5.2. Validation of the docking accuracy for beta-Ketoacyl-acyl carrier protein synthase III (FabH) receptor

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 October 2023 doi:10.20944/preprints202310.0436.v1

"Validation of the docking accuracy was done by docking the co crystalized ligand Malonyl-Coenzyme A (MLC) into the binding site of E. coli FabH receptor. The docking ligand was overlying on the native co-crystalized ligand with RMSD 0.71 Å and binding free energy was (-13.96 kcal\mol)". (Figure 5)

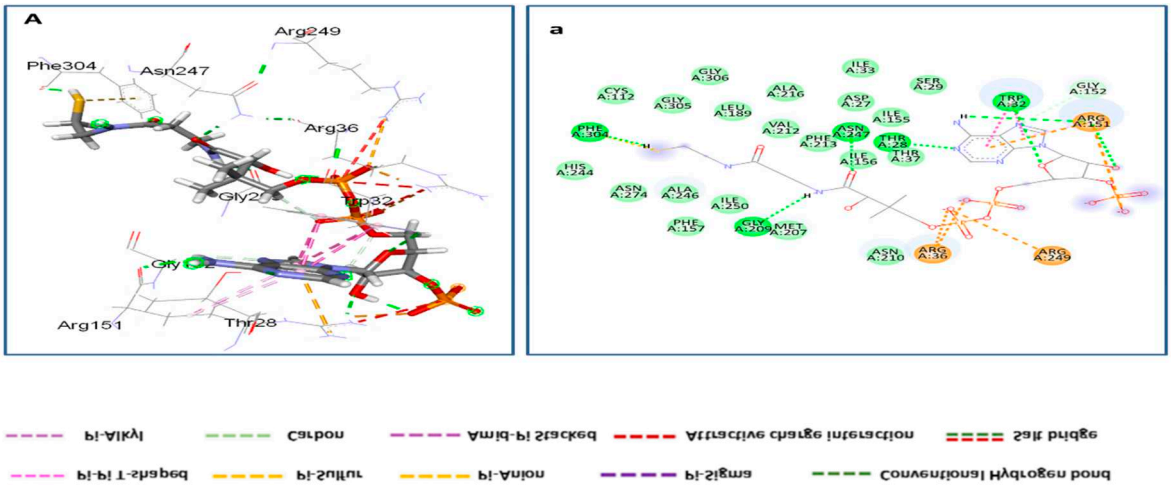


Figure 5. Docking mode of co-crystalized ligand (MLC) of FabH receptor: (A) binding sites & (a) model).

3.5.3. Validation of the docking accuracy for prostaglandin H2 synthase receptors

"Validation of the docking accuracy was done by docking the co crystalized ligand 1-(4-Iodobenzoyl)-5-Methoxy-2-Methyl Indole-3-Acetic Acid (Iodoindomethacin ligand) (IMM) into the binding site prostaglandin H2 synthase cyclooxygenase. the docking ligand was overlying on the native co-crystalized ligand with RMSD 0.97A^0 and binding free energy was (-12.53 kcal\mol)". (Figure 6)

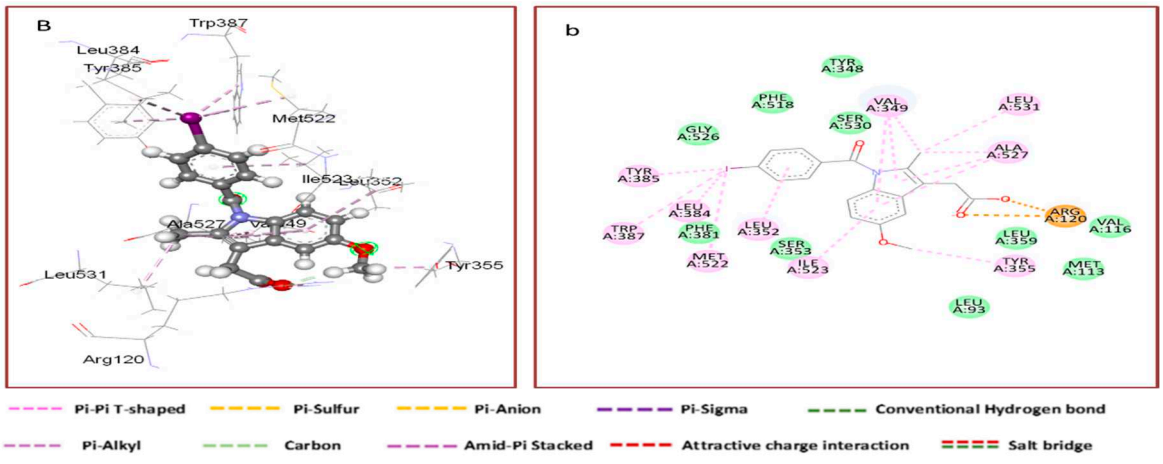


Figure 6. Docking mode of co-crystalized ligand (IMM) of Prostaglandin H2 Synthase receptor: (B) binding sites & (b) model).

Statistical analysis

"Assay results are shown as mean ± SE. Statistical analyses were carried out with Sigma Plot software (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) followed by Tukey's post test was used to assess the presence of significant differences. Differences were considered statistically significant at $p \leq 0.05$".

4. Conclusion

In the present work, a novel series of pyrazolopyrimidine derivatives (**4a–11**) were synthesized from starting material **3.** The anti-inflammatory, analgesic and antimicrobial activities of some of synthesized compounds were studied. All tested compounds exhibited anti-inflammatory compared to Indomethacin as standard drug. Also, all tested compounds exhibited analgesic activity compared to Valdecoxib as standard drug. Analysis of antimicrobial data suggested that all tested compounds significant against bacterial strains, fungal strains and yeast activities compared to Streptomycin and Erythromycin as standard drugs. Also, molecular docking study were illustrated.

Author Contributions: "R. S. M. B., M.A. and A. K. B. A. performed most of the experiments; All authors analyzed the data; All authors contributed to the pharmacological activities assays; All authors read and approved the final manuscript".

Acknowledgments: "The author acknowledges the College of Pharmacy, Taibah University and College of Pharmacy, King Saudi University, for collaboration and support to animals".

Conflicts of Interest: The authors declare that they have no Competing interest.

References

1. Katrusiak, A.; Skierska, U.; Katrusiak, A. Azido-tetrazolo tautomers of methylated azolopyridazines. *J. Mol. Str.* **2005**, 751, 65-73, https://doi.org/10.1016/j.molstruc.2005.04.025
2. Jubete, G.; Puig de la Bellacasa, R. ; Estrada-Tejedor, R.; Teixidó, J.; Borrell, J.I. Pyrido [2,3-d] pyrimidin-7 (8 H)-ones: synthesis and biomedical applications. *Molecules*. **2019**, **24**(22), 4161-4182. https://doi.org/10.3390/molecules24224161
3. Sayed, H.H.; Abbas, H.S.; Morsi, E.M.H.; Amr, A.E.; Abdelwahad, N.A.M. Antimicrobial activity of some synthesized glucopyranosyl-pyrimidine carbonitrile and fused pyrimidine systems. *Acta Pharm.* **2010**,60(40), 479-491. https:// doi.org/10.2478/v10007-010-0033-8
4. Amr A.E.; Ashraf, M.M.; Salwa, F.M.; Nagla A.A.; Hammam, A.G. Anticancer activities of some newly synthesized pyridine, pyrane, and pyrimidine derivatives. *Bioorg. Med. Chem.* **2006**, 14, 5481-5488. https://doi.org/10.1016/j.bmc.2006.04.045
5. Mohamed, S.F.; Flefel, E.M.; Amr A.E.; Abd El-Shafy, D.N. Anti-HSV-1 activity and mechanism of action of some new synthesized substituted pyrimidine, thiopyrimidine and thiazolopyrimidine derivatives. *Eur. J. Med. Chem.* **2010**,45, 1494-1501. https://doi.org/10.1016/j.ejmech.2009.12.057
6. Manzoor, S.; Almarghalani, D.A.; William James, A.; Raza, M.K.; Kausar , T.; Nayeem, S.M.; Hoda N.; Shah Z. A. Synthesis and Pharmacological Evaluation of Novel Triazole-Pyrimidine Hybrids as Potential Neuroprotective and Anti-neuroinflammatory Agents. *Pharm. Res.* **2023**, 40(1), 167-185. https://doi.org/10.1007/s11095-022-03429-1
7. Wang, H.; Cui, E.; Li, J.; Ma, X.; Jiang, X.; Du, S.; Qian, S.; Du, L. Design and synthesis of novel indole and indazole-piperazine pyrimidine derivatives with anti-inflammatory and neuroprotective activities for ischemic stroke treatment. *Eur. J. Med. Chem.* **2022**, 241, 114597. https://doi.org/10.1016/j.ejmech.2022.114597
8. Al-Omar, M.A.; Amr, A.E.; Al-Salahi, R.A. Anti-inflammatory, Analgesic, Anticonvulsant and Antiparkinsonian Activities of Some Pyridine Derivatives Using 2,6-Disubstituted Isonicotinic Acid Hydrazides. *Arch. Pharm.* **2010**,343(11-12), 648-656. https://doi.org/10.1002/ardp.201000088
9. Javed, M.A.; Jan, M.S.; Shbeer, M.A.; Al-Ghorbani, M.; Rauf, A.; Wilairatana, P.; Mannan, A.; Sadiq, A.; Farooq, U.; Rashid, U. Evaluation of pyrimidine/pyrrolidine-sertraline based hybrids as multitarget anti-Alzheimer agents: In-vitro, in-vivo, and computational studies. *Biomed. Pharmacother.* **2023**,159, 114239. https://doi.org/10.1016/j.biopha.2023.114239
10. Gangjee, A.; Adair, O.O.; Queener, S.F. Synthesis and biological evaluation of 2,4-diamino-6-(arylaminomethyl)pyrido[2,3-d]-pyrimidines as inhibitors of Pneumocystis carinii and Toxoplasma gondii dihydrofolate reductase and as antiopportunistic infection and antitumor agents. *J. Med. Chem.* **2003**, **46(23)**, 5074-5082. https://doi.org/10.1021/jm030312n
11. Elzahabi, H.S.; Nossier,E.S.; Khalifa, N.M.; Alasfoury, R.A.; El-Manawaty, M.A. Anticancer evaluation and molecular modeling of multi-targeted kinase inhibitors based pyrido[2,3-d]pyrimidine scaffold. *J. Enzyme Inhib. Med. Chem.* **2018**, **33**, 546-555. https://doi.org/10.1080/14756366.2018.1437729
12. Amr, A.E.; Ali, K.A.; Abdalla, M.M. Cytotoxic, antioxidant activities and structure activity relationship of some newly synthesized terpenoidaloxaliplatin analogs. *Eur. J. Med. Chem.* **2009**, 44, 901-907. https://doi.org/10.1016/j.ejmech.2008.05.024
13. Ibrahim, D.A.; Ismail, N.S. Design, synthesis and biological study of novel pyrido [2,3-d] pyrimidine as anti-proliferative CDK2 inhibitors. *Eur. J. Med. Chem.* **2011**,**46(12)**, 5825-5832. https://doi.org/10.1016/j.ejmech.2011.09.041

14. Yang, T.; He, H.; Ang, W.; Yang, Y.H.; Yang, J.Z.; Lin, Y.N. Syntheses and Cell-Based Phenotypic Screen of Novel 7-Amino pyrido [2,3-d] pyrimidine -6- carbonitrile Derivatives as Potential Antiproliferative Agents. Molecules, **2012**, 17(3), 2351-2366. https://doi.org/10.3390/molecules17032351
15. Mojahidi, S.; Rakib, E.; Sekkak, H.; Abouricha, S.; Benchat, N.; Mousse, H.A.; Zyad, A. Synthesis and in-vitro cytotoxic evaluation of novel pyridazin-4-one derivatives. *Arch. Pharm.* **2010**,343(5), 310-313. https://doi.org/10.1002/ardp.200900290
16. Butnariu, R.M.; Mangalagiu, I.I. New pyridazine derivatives: synthesis, chemistry and biological activity. *Bioorg. Med. Chem.* **2009**, 17, 2823-2829. https://doi.org/10.1016/j.bmc.2009.02.028
17. **Guan, L.; Sui, X.; Deng, X.Q.; Quan, Y.C.; Quan, Z.S. Synthesis and anticonvulsant activity of a new 6-alkoxy-[1,2,4]triazolo[4,3-b]pyridazine.** *Eur. J. Med. Chem.* **2010,**45(5), 1746-1752. https://doi.org/10.1016/j.ejmech.2009.12.077
18. Nagawade, R.R.; Khanna, V.V.; Bhagwat S.S.; Shinde, D.B. Synthesis of new series of 1-Aryl-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid as potential antibacterial agents. *Eur. J. Med. Chem.* **2005**,40, 1325-1330. https://doi.org/10.1016/j.ejmech.2005.05.012
19. Van Laar, M.; Volkerts, E.R.; Verbaten, M. Subchronic effects of the GABA-agonist lorazepam and the 5HT 2A/2C antagonist ritanserin on driving performance, slow wave sleep and daytime sleepiness in healthy volunteers. *Psychopharmacology*. **2001**, 154(2), 189 - 197. http://dx.doi.org /10.1007/ s002130000633
20. Amr, A.E.; Hegab, M.I.; Agami, A.; Abdalla, M.M.E. Synthesis and Reactions of Some Fused Oxazinone, Pyrimidinone, Thiopyrimidinone, and Triazinone Derivatives with a Thiophene Ring as Analgesic, Anticonvulsant, and Antiparkinsonian Agents. *Monatsh. Chem.* **2003**, 134(10), 1395-1409. https://doi.org/10.1007/s00706-003-0051-z
21. Harb, A.F.; Abbas, H.H.; Mostafa, F.H. Pyrazoles as building blocks in heterocyclic synthesis: Synthesis of pyrazolo [3,4-d]pyrimidine pyrazolo[3,4-e][1,4]diazepine, pyrazolo [3,4-d][1,2,3]triazine and pyrolo [4,3-e][1,2,4]triazolo[1,5-c]pyrimidine derivatives. *J. Iran. Chem. Soc.* **2005**, 2, 115-123. https://link.springer.com/article/10.1007/BF03247202
22. Lee, S.J.; Lee, J.S.; Hwang, K.J.; Kim, Y.K.; Kim, Y.S.; Park, N.G.; Shin, E.J.; Lee, S.H. Synthesis and characterization of phosphorescent iridium complexes of 6-chloro-3-phenylpyridazine and 3-chloro-6-(3′-methoxy-phenyl)-4-methyl-pyridazine. *Current Applied Physics.* **2005**, 5(1), 43-46. https://doi.org/10.1016/j.cap.2003.11.077
23. Hernández-Pérez, M.; Rabanal, R.M.; De la Torre, M.C.; Rodriguez, B. Analgesic, anti-inflammatory, antipyretic and haematological effects of aethiopinone, an o-naphthoquinone diterpenoid from Salvia aethiopis roots and two hemisynthetic derivatives. *planta Med.* **1995**, 61(6), 505-509. https://doi.org/10.1055/s-2006-959358
24. Ikuta, H.; Shirota, H.; kobayashi, S.; Yamagishi, Y.; Yamada, K.; Yamatsu, I.; Katayama, K. Synthesis and anti-inflammatory activities of 3- (3,5-di-tert-butyl-4-hydroxybenzylidene) pyrrolidin-2-ones. *J. Med. Chem.* **1987**, 30 (11), 1995-1998. https://pubs.acs.org/doi/abs/10.1021/jm00394a011
25. Sztaricskai, F.; Takács, E.I.; Pusztai, F.; Szabó, G.; Csípõ, I. Antiulcer effect of the N- and O-beta-D-glucopyranosides of 5-aminosalicylic acid. *Arch. Pharm.* **1999**, 332(9), 321- 326. https://pubmed.ncbi.nlm.nih.gov/10520302/
26. Sohel, M.; Sayed, A.; Azizul, I. Cytotoxic and antimicrobial activities of two new synthetic 2'-oxygenated flavones reported from Andrographis Viscosula. *J. Serb. Chem. Soc.* **2007**, 72(4), 321-329. https://doi.org/10.2298/JSC0704321M
27. Bahashwan, S.A. Pharmacological studies of some pyrimidino derivatives. *Afr. J. Pharm. Pharmacol.* **2011**, 5(4), 527-531. https://doi.org/10.5897/AJPP11.094
28. Loll, P.J.; Picot, D.; Ekabo, O.; Garavito, R.M. Synthesis and Use of Iodinated Nonsteroidal Anti-inflammatory Drug Analogs as Crystallographic Probes of the Prostaglandin H2 Synthase Cyclooxygenase Active Site. *Biochemistry*, **1996**, 35(23), 7330-7340. https://doi.org/10.1021/bi952776w
29. Qiu, X.; Janson, C.A.; Smith, W.W.; Head, M.; Lonsdale, J.; Konstantinidis, A.K. Refined structures of beta-ketoacyl-acyl carrier protein synthase III. *J. Mol. Biol.* **2001**, 307(1), 341-356. https://doi.org/10.1006/jmbi.2000.4457