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Original article

Pyrazol-3-propanoic acid derivatives as novel inhibitors of leukotriene biosynthesis in human neutrophils

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ABSTRACT

We recently presented that compounds **4a**–**b** moderately inhibited leukotriene (LT) formation in human neutrophils. For structural derivatization of **4a**–**b**, novel thirty-six title compounds were synthesized and led to more potent inhibition of LT biosynthesis in activated human neutrophils exemplified by compounds **15**, **27**–**30**, **32**–**37**, **41**, **42** with IC_{50} values in the range of 1.6–3.5 μM. Moreover, compounds **32**, **35**, **42**, **43** and **44** showed a substantial inhibition of platelet COX-1 activity with IC_{50} of 2.5, 0.041, 0.3, 0.9 and 0.014 μM, respectively, leading up to dual acting inhibitors. On the basis of their high potency in cellular environment, these straightforward pyrazole-3-propanoic acid derivatives may possess potential in the design of more potent compounds for intervention with inflammatory and allergic diseases.

1. Introduction

It has been well established that leukotrienes (LTs) formed from arachidonic acid (AA) by the 5-lipoxygenase (5-LO) pathway play important roles in various biological processes such as bronchoconstriction, increased vascular permeability, and vasoconstriction of coronary arteries [1]. 5-LO is the key enzyme in the conversion of AA to LTA_4 in two successive steps. First, oxygenation of AA forms 5(S)-hydroperoxy-6,8,11,14(E,Z,Z,Z)-eicosatetraenoic acid (5-HPETE), and then, dehydration leads to the unstable epoxide LTA_4 which is subsequently converted to either LTB_4 by LTA_4 hydrolase or to cysteinyl-LTs (LTC_4, D_4 and E_4) through conjugation with glutathione catalyzed by LTC_4 synthases [2]. In intact cells, the 5-LO-mediated formation of LTA_4 from AA also requires a concomitant involvement of the 5-LO-activating protein (FLAP) that transfers AA to 5-LO for efficient metabolism [3]. LTB_4 acts as a high affinity ligand for G protein-coupled receptors (GPCR), namely BLT_1 and BLT_2 which stimulates the inflammatory response as well as neutrophil and eosinophil chemotaxis [4]. The cysteinyl LTs also activate GPCRs such as $CysLT_1$ and $CysLT_2$ causing bronchoconstriction, airway edema and mucus secretion [1]. Therefore, inhibition of LT formation represent valuable target for the therapy of inflammatory ailments like asthma, allergic rhinitis, atherosclerosis as well as certain types of cancers [5,6]. Today, while cysteinyl-LT receptor antagonists (i.e. montelukast and zafirlukast) are successfully applied for asthma therapy, the development of LT biosynthesis inhibitors is little advanced [7–10] and thus far zileuton is the only 5-LO inhibitor currently in clinical use [11] (Fig. 1).

Recently, we have presented that 1,5-diarylpyrazol-3-propanoic acids (**4a**–**b** in Fig. 1) inhibit LTB_4 biosynthesis in human neutrophils with IC_{50} values of 12–14 μM [12]. In order to obtain more potent derivatives, we aimed at modifying structural features of **4a**–**b**. In particular, the structural changes in the acidic part of compounds under study did yield substantial improvement of LTB_4 biosynthesis inhibition in comparison with the previously prepared derivatives **4a**–**b**. We hereby demonstrate that preparing certain amides and esters of **4a**–**b** may lead to potent inhibition of LT biosynthesis in intact cells without interfering the 5-LO activity since none of the compounds resulted in inhibition of 5-LO enzyme in cell-free systems. Our results present a novel pharmacophore for suppression of LT formation in intact cells, essentially lacking inhibitory effects on other important enzymes within the AA cascade such as 5/12/15-LO, COX-1/2, and microsomal prostaglandin E_2 synthase (mPGES-1).

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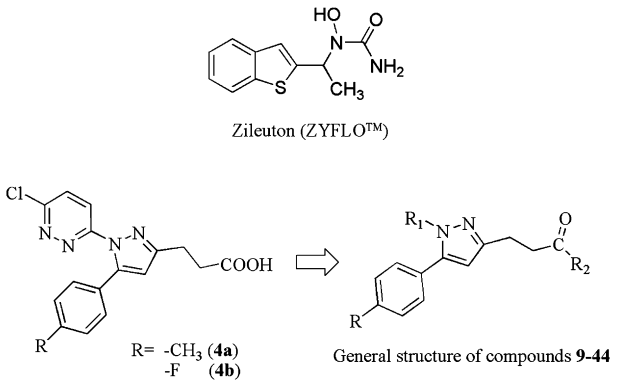


Fig. 1. Structure of zileuton, the lead compounds (**4a**–**b**) and general structure of the synthesized compounds.

2. Results and discussion

2.1. Chemistry

The target compounds were prepared according to the reaction sequence shown in Fig. 2. The synthesis of 6-phenyl-4,6-dioxohexanoic acid derivatives (**1a**–**c**) was achieved by condensation of the commercially available acetophenones with succinic anhydride in the presence of a strong base as published previously [12]. The regioselective synthesis of 1,5-diarylpyrazole derivatives (**4a**–**c** to **6a**–**c**) was conveniently accomplished by condensation of the requisite diketone with the hydrochloride salt of the arylhydrazine derivative (**4**–**6**) in methanol/triethylamine with slight modifications of the previously published procedures [12–14]. For the quinoline derivatives (**7a**–**c**), the reaction was carried out with 2-hydrazinoquinoline and diketones in the absence of triethylamine. Nucleophilic substitution reaction of 6-chloropyridazine derivatives (**4a**–**c**) with sodium methoxide in methanol yielded 6-methoxypyridazines (**8a**–**c**). The first series of amide derivatives (**9**–**11**, **15**–**17**, **22**–**24**) were obtained by coupling of acids (**4a**–**4c** and **6a**) with various amines using ethyl chloroformate as the carboxyl group activator in the presence of triethylamine. The same

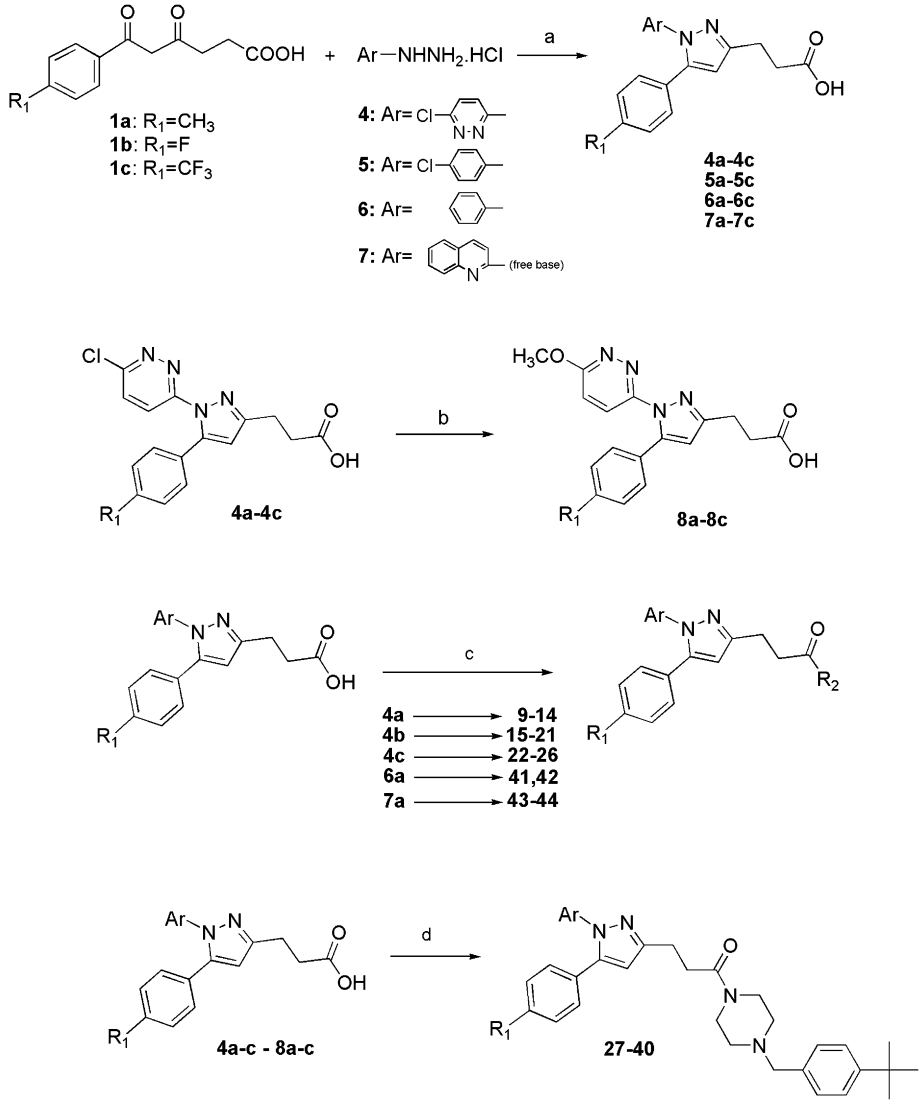


Fig. 2. Synthesis of ester and amide derivatives of 1,5-diaryl-pyrazol-3-propanoic acids (**9**–**44**). Reagents and conditions: (a) Et_3N, MeOH, reflux; (b) *i.* $NaOCH_3$, MeOH, reflux *ii.* H^+; (c) Amine derivatives, ethyl chloroformate, Et_3N, CH_2Cl_2, rt/Amine or Phenol derivatives, EDC, DMAP, CH_2Cl_2, rt; (d) *tert*-butylbenzylpiperazine, EDC, DMAP, CH_2Cl_2, rt.

acids also coupled with various phenols under the activation of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in the presence of 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2 to afford the ester derivatives (**12**–**14**, **18**–**21**, **25**, **26** and **42**–**44**). The second series of amide derivatives (**27**–**41**) were synthesized by the reaction of acid derivatives (**4**–**8a**–**c**) with corresponding amines also using EDC and DMAP in CH_2Cl_2. Compounds were purified by automated flash chromatography and checked for purity with UPLC before being tested in biological assays (purity was >97%). The structures of these compounds were confirmed by high resolution mass spectrometry (HRMS), IR and ^{1}H-NMR spectral data.

Our strategy to optimize the activity in our series was concentrated on three distinct areas of the lead molecules, 3-(1-(6-chloropyridazin-3-yl)-5-(4-methyl- or 4-fluorophenyl)-1*H*-pyrazol-3-yl)propanoic acid (**4a**–**b**), to deduce structure-activity relationships (SARs). The areas that we addressed were (i) modification of the propanoic acid side-chain, (ii) replacement of the chloropyridazin of the central pyrazole with different aryl groups, and (iii) incorporation of different *p*-substituents on the C5-phenyl of the central pyrazole ring. Together, by incorporation of distinct chemical functionalities by preparing amide and ester derivatives of **4a**–**b**, we first initiated the synthesis of pyrazole derivatives (**9**–**26**) as anti-LT agents (Table 1).

2.2. Analysis of inhibition of LT synthesis and structure-activity relationships

For LT biosynthesis, AA must be released from membrane phospholipids by $cPLA_2$ which is then converted to LTA_4 by the action of 5-LO aided by FLAP [2]. The activation of 5-LO occurs as a result of Ca^{2+} mobilization and/or 5-LO phosphorylation by protein kinases, eventually leading to an association of 5-LO with membranes in close vicinity to FLAP [2]. For a thorough analysis of the inhibitory potential of the test compounds, two different test systems were applied, that is, a cell-based assay using human neutrophils and a cell-free assay using human recombinant 5-LO. In the cell-based assay various mechanisms aside from direct interference with 5-LO enzyme may cause suppression of LT formation such as inhibition of AA release, antagonism of FLAP, suppression of 5-LO-activating kinases or of Ca^{2+} mobilization, and inhibition of 5-LO membrane association [2].

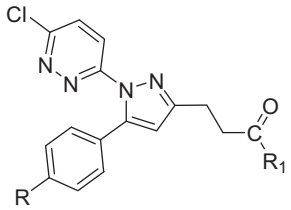
The initial screening of **9**–**26** for their ability to inhibit 5-LO product formation in isolated human neutrophils was performed at a concentration of 10 μM. Neutrophils were challenged with Ca^{2+} ionophore A23187 that causes substantial formation of 5-LO products as a result of elevated intracellular Ca^{2+} concentration and thus release of AA as substrate for 5-LO [15,16]. However, diminished 5-LO product formation may thus not necessarily result from the inhibition of 5-LO but could also arise from the inhibition of $cPLA_2$ leading to suppressed levels of AA. Therefore, neutrophils were supplemented with exogenous AA (20 μM) to circumvent the necessity of $cPLA_2$– mediated endogenous AA supply. In addition, compounds were also tested in a cell-free system using supernatants of lysates of *Escherichia coli* expressing human recombinant 5-LO (or ATP-affinity purified 5-LO), thereby assessing the direct inhibition of crude enzymatic activity of 5-LO [17]. The iron ligand-type 5-LO inhibitor *N*-(3-phenoxycinnamyl)-acetohydroxamic acid (BWA4C) [18] which is closely related to the clinically developed zileuton [19], was used as reference compound. BWA4C suppressed 5-LO product synthesis in the cell-based assay with IC_{50} value of 0.06 ± 0.01 μM, while the IC_{50} value in the cell-free assay was 0.038 ± 0.04 μM, respectively. Zileuton is known to result in inhibition of 5-LO product synthesis in PMNL assay with IC_{50} value of about 0.8–1 μM [19,20].

First, the modifications of 1,5-diarylpyrazole-3-propanoic acid structure were introduced basically to obtain compounds with different physicochemical characteristics while varying the substituent at the phenyl ring of C5-pyrazole and keeping the chloropyridazine ring at the N1-pyrazole (compounds **9**–**26** in Table 1). Among the tested compounds, only the ester derivative formed with *i*-propylphenol (**12**) as well as the amide derivatives of *tert*-butylbenzylpiperazine (**15**) and of ethyl piperidinecarboxylate (**23**) showed potent inhibition of 5-LO product synthesis in the cell-based assay. Interestingly, the other amide and ester analogs failed in inhibition of 5-LO product synthesis. For example, linking the carboxyl group with piperidinecarboxamide (**22**) instead of more lipophilic ethyl piperidinecarboxylate (**23**) was detrimental for inhibitory activity. With regard to piperazine amides, while the lipophilic bulky *tert*-butylbenzyl substituent was well tolerated (**15**), the activity with more polar piperazine derivatives (**9**, **11**, **16**, **24**) was not evident. For the ester analogs, the most potent inhibitor was the *i*-propylphenyl derivative (**12**) and keeping small lipophilic *p*-substituents such as *tert*-butyl (**18**) or *cyclo*-pentyl (**25**) in the phenol moiety still governed inhibition of cellular 5-LO product formation. However, the replacement of *i*-propyl by electron withdrawing substituents (trifluoromethoxy (**14**), thiophene (**19**)) or by heterocyclic ring systems (imidazole (**21**) and pyrrole (**26**)) generally diminished the inhibitory activity. Since the compounds **12** and **15** were the only derivatives that elicited more than 50% inhibition at 10 μM, the IC_{50} values were further calculated from concentration-response curves and determined as 8 and 1.6 μM, respectively.

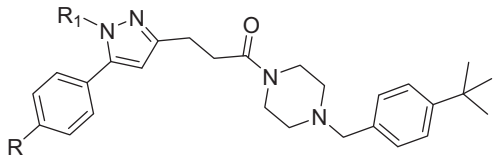
It should be noted that, regardless of each and every modification, none of the compounds demonstrated substantially inhibitory activity against 5-LO activity in a cell-free assay, whereas the reference compound BWA4C efficiently suppressed 5-LO activity under the same conditions as expected. This suggests that the inhibition of 5-LO product formation in intact neutrophils by the active test compounds did not result from a direct interaction with the 5-LO enzyme.

On the basis of the results from the initial screening and also considering the piperazine moiety as a privileged structure with drug-like properties, compound **15** was selected for further modifications. The chloropyridazine core in **15** was systematically modified with different aromatic counterparts such as chlorophenyl (**29**–**31**), phenyl (**32**–**34**), quinoline (**35**–**37**) and methoxypyridazine (**38**–**40**) while varying the *p*-substituent on the phenyl moiety of C5-pyrazole (Table 1). While replacement of chloropyridazine in **15** with a phenyl ring (**33**) slightly reduced the potency, the phenyl substitution in **32** and **34** versus respective chloropyridazine derivatives (**27**–**28**) did not change the efficiency as seen from the calculated IC_{50} values. When the lipophilicity was increased by incorporation of chlorophenyl (**29**–**31**) or quinoline (**35**–**37**), the compounds retained the inhibitory activity although efficiency was slightly reduced compared to the corresponding chloropyridazine derivatives (**15**, **27** and **28**). Finally, substituting the chloropyridazine moiety by methoxypyridazine (**38**–**40**) caused a decrease in the potency. Note that all these structural modifications also failed to generate compounds that inhibited the activity of 5-LO in the cell-free assay (Table 1). Detailed concentration-response studies of active derivatives in the cell-based assay (using neutrophils challenged with A23187 plus 20 μM AA), revealed IC_{50} values in the range of 1.6–10 μM. In conclusion, among all compounds, the *tert*-butylbenzylpiperazine amide derivative with a vicinal 6-chloropyridazine- and 4-fluorophenyl-substitution about a central pyrazole was the most efficient one (**15**, IC_{50} = 1.6 μM) with a potency close to the clinical drug zileuton (IC_{50} = 0.8–1 μM) under the same assay conditions [19]. Moreover, the IC_{50} values of all other closely related

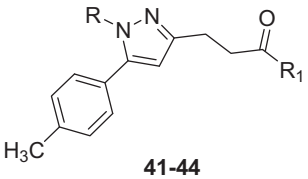
Table 1
Inhibition of 5-LO product formation by test compounds (**9**–**44**) in (**A**) intact neutrophils and in (**B**) a cell-free assay[a] at 10 μM. Data are given as mean ± SEM of remaining % 5-LO activity versus vehicle (0.3% DMSO), $n = 3–5$ $^{*}p < 0.05$, $^{**}p < 0.01$.



9-26



27-40



41-44

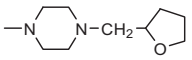
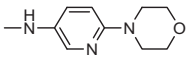
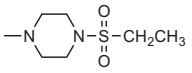
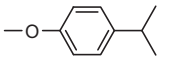
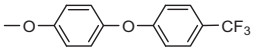
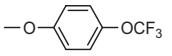
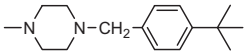
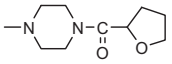
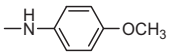
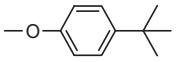
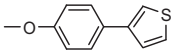
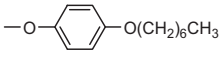
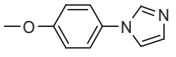
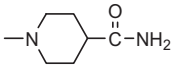
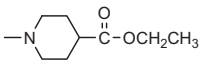
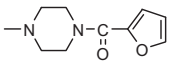
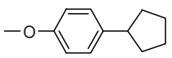
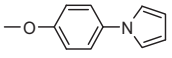
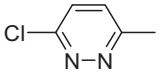
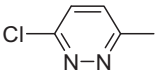
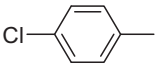
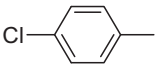
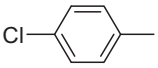
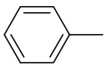
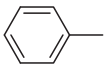
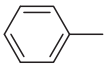
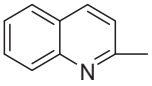
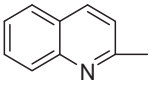
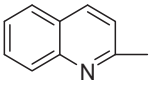
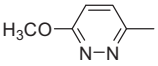
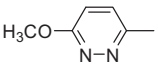
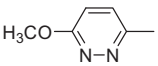
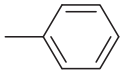
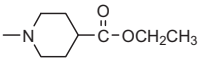
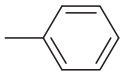
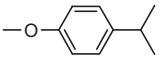
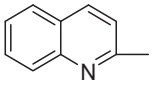
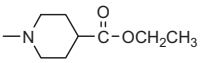
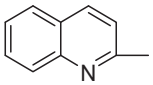
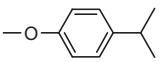
Compd.	*R*	R_1	**A**	**B**	IC_{50} [μM][b]
9	—CH_3		86.0 ± 11.5	105.6 ± 16.4	ND
10	—CH_3		106.2 ± 6.4	90.0 ± 11.0	ND
11	—CH_3		96.3 ± 5.0	93.6 ± 6.9	ND
12	—CH_3		46.4 ± 9.3	92.7 ± 12.7	8.0
13	—CH_3		86.5 ± 4.2	95.0 ± 10.9	ND
14	—CH_3		70.5 ± 12.3	115.8 ± 6.5	ND
15	—F		23.4 ± 4.6	55.3 ± 10.5	1.6
16	—F		95.0 ± 5.4	90.8 ± 9.6	ND
17	—F		88.3 ± 7.7	91.6 ± 14.9	ND
18	—F		67.3 ± 15.8	92.7 ± 4.3	ND
19	—F		90.8 ± 6.6	83.4 ± 24.9	ND
20	—F		90.1 ± 7.7	86.5 ± 8.7	ND
21	—F		111.4 ± 4.4	71.7 ± 10.9	ND
22	—CF_3		104.3 ± 6.3	102.9 ± 17.3	ND
23	—CF_3		62.0 ± 7.9	72.6 ± 9.2	15.0
24	—CF_3		81.8 ± 10.1	125.5 ± 22.7	ND
25	—CF_3		77.2 ± 6.9	92.0 ± 6.3	ND
26	—CF_3		94.1 ± 11.3	90.8 ± 14.9	ND
27	—CH_3		14.6 ± 0.5	107.4 ± 25.0	2.8

Table 1 (*continued*)

Compd.	*R*	R_1	**A**	**B**	IC_{50} [μM][b]
28	—CF_3		17.4 ± 8.1	88.9 ± 28.4	2.9
29	—CH_3		27.7 ± 8.4	111.3 ± 23.6	2.9
30	—F		13.2 ± 3.4	143.3 ± 3.5	2.6
31	—CF_3		43.9 ± 9.9	137.8 ± 37.5	8.0
32	—CH_3		13.5 ± 5.8	85.6 ± 27.8	2.7
33	—F		3.7 ± 2.2	78.5 ± 20.4	2.5
34	—CF_3		12.7 ± 4.4	109.3 ± 25.2	2.9
35	—CH_3		16.4 ± 3.1	51.5 ± 6.1	3.5
36	—F		10.4 ± 5.2	114.1 ± 31.6	2.8
37	—CF_3		44.5 ± 16.3	87.3 ± 24.8	3.0
38	—CH_3		27.1 ± 4.6	86.1 ± 8.2	3.3
39	—F		50.1 ± 14.1	69.2 ± 3.5	> 10
40	—CF_3		36.2 ± 15.4	56.1 ± 21.8	8
41			6.7 ± 3.0	102.5 ± 11.6	2.4
42			20.4 ± 0.9	95.3 ± 6.2	2.0
43			25.8 ± 0.9	69.9 ± 9.9	4.0
44			65.4 ± 6.6	79.6 ± 12.3	>10

[a] Values for 5-LO product formation in intact human neutrophils challenged with 2.5 μM ionophore plus 20 μM arachidonic acid (A) and under cell-free conditions (supernatants of lysates of *E. coli* expressing human recombinant 5-LO incubated with 20 μM arachidonic acid (B) as percentage of control at 10 μM inhibitor concentrations are expressed as mean ± SE. BWA4C was used as reference compound at a concentration of 0.3 μM leading to 13.0 ± 3.8% and 31.5 ± 7.9% remaining 5-LO activity in the cell-based and the cell-free assay, respectively.

[b] IC_{50} values for LT formation of selected compounds were obtained in intact neutrophils challenged with 2.5 μM ionophore plus 20 μM arachidonic acid.

Table 2
Comparison of the selectivity of some test compounds for related enzymes in the arachidonic acid cascade. Compounds are tested at 10–30 μM concentrations and data are given as mean ± SEM of % remaining activity compared to vehicle (IC_{50} values, μM, are given in parentheses).

Compd.	PMNL[a]	Cell-free[b,c]	COX-1[d]	COX-2[d]	mPGES-1[e]	12-LO[f]	15-LO[f]
12	46.4 ± 9.3 (8)	92.7 ± 12.7[b] 83.4 ± 15.9[c] (>10)	51.1 ± 3.9 (>10)	99.9 ± 17.9 (>10)	91.4 ± 4.6 (>10)	77.1 ± 29.2 (>10)	n.d.
15	23.4 ± 4.6 (1.6)	55.3 ± 10.5[b] 102.8 ± 11.2[c] (>10)	82.9 ± 3.2 (>10)	84.2 ± 9.2 (>10)	75.6 ± 7.0 (>10)	102.9 ± 13.4 (>10)	175 ± 43 (>10)
23	62.0 ± 7.9 (>10)	72.6 ± 9.2[b] 84 ± 13.8[c] (>10)	102.2 ± 24.4 (>10)	78.6 ± 21.5 (>10)	103.0 ± 6.1 (>10)	90.4 ± 25.4 (>10)	71 ± 45 (>10)
32	13.5 ± 5.8 (2.7)	85.6 ± 27.8[b] 62.9 ± 17.9[c] (>10)	21.0 ± 2.9 (2.5)	97.0 ± 11.7 (>10)	84.6 ± 2.5 (>10)	118.4 ± 32.4 (>10)	261 ± 49 (>10)
35	16.4 ± 3.1 (3.5)	51.5 ± 6.1[b] 115.1 ± 10.5[c] (>10)	8.2 ± 1.0 (0.041)	79.0 ± 29.2 (>10)	75.0 ± 4.2 (>10)	110.6 ± 23.7 (>10)	145.0 ± 22 (>10)
41	6.7 ± 3.0 (2.4)	102.5 ± 11.6[b] 79.5 ± 13.1[c] (>10)	57.1 ± 12.1 (>10)	86.3 ± 9.1 (>10)	82.6 ± 3.5 (>10)	98.0 ± 6.1 (>10)	196 ± 30 (>10)
42	20.4 ± 0.9 (2.0)	95.3 ± 6.2[b] 92.6 ± 18.0[c] (>10)	10.6 ± 1.3 (0.3)	111.1 ± 26.0 (>10)	108.2 ± 7.2 (>10)	67.2 ± 0.2 (>10)	124 ± 22 (>10)
43	25.8 ± 0.9 (4.0)	69.9 ± 9.9[b] 55.9 ($n = 1$)[c] (>10)	15.9 ($n = 1$) (0.9)	66.3 ± 9.1 (>10)	84.0 ± 4.1 (>10)	91.9 ± 27.6 (>10)	149.0 ± 18 (>10)
44	65.4 ± 6.6 (>10)	79.6 ± 12.3[b] 86.1 ± 12.9[c] (>10)	16.9 at 1 μM (0.014)	97.0 ($n = 1$) (>10)	88.0 ± 4.4 (>10)	67.8 ± 15.9 (>10)	92.0 ± 14 (>10)

[a] Values for 5-LO product formation at 10 μM in intact human neutrophils.
[b] under cell-free conditions with supernatants of lysates of *E. coli* expressing human recombinant 5-LO.
[c] under cell free conditions with purified 5-LO using 20 μM AA as substrate.
[d] COX residual activity at 10 μM [% 12-HHT of control].
[e] mPGES-1 residual activity at 10 μM [% PGE_2 of control].
[f] 15- and 12-LO residual activity at 30 μM, 15-H(P)ETE was analyzed as a product of 15-LO and 12-H(P)ETE as a product of 12-LO. n.d. = not done.

derivatives were also found in a narrow and comparable range (IC_{50} of between 1.6 and 3.5 μM except for **12**, **23**, **31**, **39**, **40**, **43** and **44**).

Since amidation of the carboxylic acid side chain with ethyl piperidinecarboxylate (**23**) and esterification with *i*-propylphenol (**12**) in chloropyridazine series might contribute to the efficiency for inhibition of 5-LO product synthesis in intact neutrophils, we next evaluated the respective amide and ester analogs of phenyl and quinoline substituted core structures (**6a** and **7a**) in place of chloropyridazine. As shown in Table 1, phenyl substituted compounds (**41**–**42**) also significantly suppressed 5-LO product synthesis at 10 μM, whereas quinoline substitution (**43**–**44**) was not very effective. Apparently, combination of N1-phenyl and C5-*p*-tolyl substitution at a central pyrazole and amidification with ethyl piperidinecarboxylate or esterification with *i*-propylphenol even increased the potency leading to **41** (IC_{50} = 2.4 μM) and **42** (IC_{50} = 2.0 μM) with respect to corresponding chloropyridazine derivatives (**23,** IC_{50} = 15 μM and **12,** IC_{50} = 8 μM).

Many researchers over the last decades have reported on compounds with dual action that inhibit both 5-LO and other enzymes involved in AA cascade [21–23]. Therefore, nine selected compounds were also tested for enzyme selectivity for related enzymes in the AA cascade such as COXs, mPGES-1, 12-LO and 15-LO (Table 2). As a result, none of the compounds were found to inhibit 12- or 15-LO up to 30 μM as measured by the formation of 12-HETE or 15-HETE in the cell-based assay. In addition, none of them demonstrated inhibition of mPGES-1 activity at 10 μM measured in microsomal preparations of IL-1β-stimulated A549 cells. The activity of COX-2 in IL-1β-stimulated A549 cells was not significantly influenced at this concentration as well. However, incorporation of phenyl (**32** and **42)** or quinoline (**35**, **43** and **44**) in place of chloropyridazine ring caused a potent inhibition of platelet COX-1 activity with IC_{50} values of 2.5, 0.3, 0.041, 0.9 and 0.014 μM, respectively, in which the two of them were more potent than the used control inhibitor indomethacin (IC_{50} = 0.07 μM) [12].

3. Conclusions

LTs are important mediators derived from AA metabolism by the 5-LO pathway and have been shown to mediate various inflammatory reactions [1]. The clinical efficacy of the inhibition of LT biosynthesis has been proven by the 5-LO iron-ligand inhibitor zileuton, which is currently the only approved antiasthmatic agent that inhibits LT biosynthesis by direct interference with 5-LO. However, zileuton carries certain disadvantages including drug–-drug interactions, frequent dosing and hepatotoxic side-effects [24]. Moreover, the utilization of anti-LT compounds in treating the inflammatory component of cardiovascular disease comprising atherosclerosis, myocardial infarction and stroke has only been seriously conceived in the past few years [25]. In addition, LO and COX metabolic pathways are becoming visible as key regulators of cell proliferation and neo-angiogenesis and inhibitors of both pathways are being investigated as potential anticancer drugs [26,27].

Our results clearly show that derivatization of 1,5-diarylpyrazol-3-propanoic acid scaffold into lipophilic amides and esters, exemplified in this work, act as suppressors of LT biosynthesis in intact cells without remarkable direct inhibition of the 5-LO enzyme in cell-free assays, exemplified by **15**, **27**–**30**, **32**–**37**, **41**–**42** with IC_{50} values in the range of 1.6–3.5 μM. More detailed analysis revealed no or minor inhibition of the related 12/15-LOs, COX enzymes or mPGES-1 by the chloropyridazine derivatives. Substitution of chloropyridazine with more lipophilic phenyl or quinoline rings in analogous compounds resulted in the potent inhibition of COX-1 activity in platelets besides inhibition of LT biosynthesis thereby acting as dual inhibitors. Taken together, we present a novel pharmacophore for interfering with cellular LT formation, and the lead compounds may possess potential for developing novel molecules for treatment of diseases requiring anti-LT therapy.

4. Experimental section

4.1. Chemistry

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$or DMSO-d_6 on a Varian Mercury 400 MHz High Performance Digital FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). High resolution mass

spectra data (HRMS) were collected in-house using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in either ESI (+) or ESI (−) methods, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected (Schorpp Geaetetechnik, Germany). IR spectra were obtained using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory and only carbonyl stretching frequencies were given. Flash chromatography was performed with a Combiflash®Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using hexane-ethyl acetate or dichloromethane-methanol solvent gradients. The purity of the final compounds was determined to be >97% by UPLC with UV detector. Compounds **1a**–**c**, **4**, **4a**–**c** and **8a**–**c** were synthesized as described previously [12]. 2-Hydrazinoquinoline (**7**) was synthesized by the reaction of 2-chloroquinoline with hydrazine hydrate [28].

4.2. General procedure for the synthesis of *5*–*7a*–*c*

To a solution of 6-aryl-4,6-diketohexanoic acid (**1a**–**c**) (2.1 mmol) in methanol (15 ml) containing Et_3N (4.2 mmol), 4-chlorophenylhydrazine hydrochloride (**5**), phenylhydrazine hydrochloride (**6**) or 2-hydrazinoquinoline (**7**) (2.1 mmol) was added and heated at reflux for 4 h (in the case of **7**, the reaction was carried out in the absence of Et_3N). The resulting solution was evaporated in vacuo, taken up in ethyl acetate and washed with 1N HCl (30 ml) and water (30 ml). The organic phase was extracted with 5% $NaHCO_3$ (3 × 30 ml) and aqueous phase containing sodium salt of the product was acidified with 6N HCl. The precipitate was collected by filtration and washed with water. The crude product was purified by flash chromatography or by recrystallization from the appropriate solvent.

4.2.1. 3-[1-(4-Chlorophenyl)-5-(4-methylphenyl)-1H-pyrazol-3-yl] propanoic acid (*5a*)

Prepared from **1a** and **5**, purified by flash chromatography using CH_2Cl_2:methanol (95:5); yield 44%; Mp 138.5–139.5 °C; IR (ATR): 1718 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.30 (s, 3H), 2.64 (t, 2H, J = 7.6 Hz), 2.86 (t, 2H, J = 7.6 Hz), 6.46 (s, 1H), 7.10 (d, 2H, J = 8.0 Hz), 7.18 (d, 2H, J = 8.0 Hz), 7.23 (m, 2H), 7.45 (m, 2H), 12.1 (bs, 1H); HRMS (m/z): $[M + H]^+$calcd for $C_{19}H_{18}ClN_2O_2$, 341.1057; found, 341.1073.

4.2.2. 3-[1-(4-Chlorophenyl)-5-(4-fluorophenyl)-1H-pyrazol-3-yl] propanoic acid (*5b*)

Prepared from **1b** and **5**, purified by recrystallization from hexane:ethyl acetate; yield 53%; Mp 126–127 °C; IR (ATR): 1719 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.61 (t, 2H, J = 7.4 Hz), 2.83 (t, 2H, J = 7.4 Hz), 6.48 (s, 1H), 7.17–7.24 (m, 6H), 7.42 (m, 2H), 12.1 (bs, 1H); HRMS (m/z): $[M-H]^-$calcd for $C_{18}H_{13}ClFN_2O_2$, 343.0650; found, 343.0663.

4.2.3. 3-{1-(4-Chlorophenyl)-5-[4-(trifluoromethyl)phenyl]-1H-pyrazol-3-yl}propanoic acid (*5c*)

Prepared from **1c** and **5**, purified by recrystallization from hexane:ethyl acetate; yield 30%; Mp 168–169.3 °C; IR (ATR): 1714 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.66 (t, 2H, J = 7.6 Hz), 2.89 (t, 2H, J = 7.6 Hz), 6.66 (s, 1H), 7.27 (d, 2H, J = 8.4 Hz), 7.44 (d, 2H, J = 8.4 Hz), 7.48 (d, 2H, J = 8.4 Hz), 7.75 (d, 2H, J = 8.4 Hz), 12.1 (bs, 1H); HRMS (m/z): $[M + H]^+$calcd for $C_{19}H_{15}ClF_3N_2O_2$, 395.0774; found, 395.0768.

4.2.4. 3-[5-(4-Methylphenyl)-1-phenyl-1H-pyrazol-3-yl]propanoic acid (*6a*)

Prepared from **1a** and **6**, purified by recrystallization from hexane:ethyl acetate; yield 71%; Mp 145.5–146.4 °C; IR (ATR): 1704 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.28 (s, 3H), 2.65 (t, 2H, J = 7.6 Hz), 2.87 (t, 2H, J = 7.6 Hz), 6.45 (s, 1H), 7.08 (d, 2H, J = 8.4 Hz), 7.14 (d, 2H, J = 8.0 Hz), 7.22 (m, 2H), 7.30–7.40 (m, 3H); HRMS (m/z): $[M + H]^+$calcd for $C_{19}H_{19}N_2O_2$, 307.1447; found, 307.1433.

4.2.5. 3-[5-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-3-yl]propanoic acid (*6b*)

Prepared from **1b** and **6**, purified by recrystallization from hexane:ethyl acetate; yield 51%; Mp 142.3–142.8 °C; IR (ATR): 1717 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.63 (t, 2H, J = 7.6 Hz), 2.88 (t, 2H, J = 7.6 Hz), 6.50 (s, 1H), 7.17–7.26 (m, 6H), 7.33–7.41 (m, 3H); HRMS (m/z): $[M + H]^+$calcd for $C_{18}H_{16}FN_2O_2$, 311.1196; found, 311.1183.

4.2.6. 3-{1-Phenyl-5-[4-(trifluoromethyl)phenyl]-1H-pyrazol-3-yl} propanoic acid (*6c*)

Prepared from **1c** and **6**, purified by flash chromatography using CH_2Cl_2:methanol (95:5); yield 48%; Mp 105.5–106.3 °C; IR (ATR): 1718 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.67 (t, 2H, J = 7.6 Hz), 2.90 (t, 2H, J = 7.6 Hz), 6.65 (s, 1H), 7.25 (m, 2H), 7.37–7.44 (m, 5H), 7.72 (d, 2H, J = 8.8 Hz), 12.1 (bs, 1H); HRMS (m/z): $[M + H]^+$calcd for $C_{19}H_{16}F_3N_2O_2$, 361.1164; found, 361.1165.

4.2.7. 3-[5-(4-Methylphenyl)-1-quinolin-2-yl-1H-pyrazol-3-yl] propanoic acid (*7a*)

Prepared from **1a** and **7**, purified by recrystallization from hexane:ethyl acetate, yield 43.5%; Mp 128–128.5 °C; IR (ATR): 1703 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H,), 2.87 (t, 2H, J = 7.6 Hz), 3.12 (t, 2H, J = 7.6 Hz), 6.38 (s, 1H), 7.08 (d, 2H, J = 8 Hz), 7.19 (d, 2H, J = 8 Hz), 7.49 (m, 2H), 7.65 (t, 1H J = 7.2), 7.77 (d, 1H, J = 8 Hz) 7.85 (d, 1H, J = 8.4 Hz), 8.11 (d, 1H, J = 8.4 Hz), 10.92 (bs, 1H); ^{13}C NMR ($CDCl_3$): δ 21.57, 23.65, 33.82, 108.60, 117.52, 126.87, 127.20, 127.65, 128.39, 129.06, 129.12, 129.40, 130.30, 138.44, 138.54, 145.30, 146.86, 151.19, 153.35, 178.49. HRMS (m/z): $[M + H]^+$calcd for $C_{22}H_{20}N_3O_2$, 358.1556; found, 358.1539.

4.2.8. 3-[5-(4-Fluorophenyl)-1-quinolin-2-yl-1H-pyrazol-3-yl] propanoic acid (*7b*)

Prepared from **1b** and **7**, purified by recrystallization from hexane:ethyl acetate, yield 26%; Mp 159–160.3 °C; IR (ATR): 1696 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.71 (t, 2H, J = 7.4 Hz), 2.93 (t, 2H, J = 7.4 Hz), 6.58 (s, 1H), 7.19 (m, 2H), 7.37 (m, 2H), 7.44 (d, 1H, J = 8.0 Hz), 7.57 (m, 1H), 7.69 (m, 1H), 7.92 (d, 1H, J = 8.8 Hz), 8.00 (d, 1H, J = 7.2), 8.51 (d, 1H, J = 8.8 Hz); HRMS (m/z): $[M + H]^+$calcd for $C_{21}H_{17}FN_3O_2$, 362.1305; found, 362.1295.

4.2.9. 3-{1-Quinolin-2-yl-5-[4-(trifluoromethyl)phenyl]-1H-pyrazol-3-yl}propanoic acid (*7c*)

Prepared from **1c** and **7**, purified by flash chromatography using CH_2Cl_2:methanol (98:2); yield 48.5%; Mp 166.2–167.2 °C; IR (ATR): 1689 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.73 (t, 2H, J = 7.4 Hz), 2.96 (t, 2H, J = 7.4 Hz), 6.71 (s, 1H), 7.34 (d, 1H, J = 8.4 Hz), 7.55–7.72 (m, 6H), 8.00 (m, 2H), 8.54 (d, 1H, J = 8.8 Hz); HRMS (m/z): $[M + H]^+$calcd for $C_{22}H_{17}F_3N_3O_2$, 412.1273; found, 412.1266.

4.3. General procedure for the synthesis of *9*–*26*

Synthesis of amide derivatives (Procedure 1) Compounds **4a**–**c** (1.27 mmol), Et_3N (1.4 mmol) and ethyl chloroformate (1.27 mmol) in CH_2Cl_2 (10 ml) was stirred at −5 °C for 1 h. The solution of the appropriate amine derivative (1.4 mmol) and triethylamine

(1.4 mmol) in CH_2Cl_2 (5 ml) was slowly added to the reaction mixture, and stirred at room temperature overnight. It was diluted with CH_2Cl_2 and washed with 1N HCl (3 × 25 ml), 5% $NaHCO_3$ solution (3 × 25 ml) and brine. After drying over Na_2SO_4, solvent was evaporated off under reduced pressure to dryness. The crude product was purified by flash chromatography or by recrystallization from the appropriate solvent.

Synthesis of ester derivatives (Procedure 2) To a mixture of the appropriate acid derivative (**4a**–**c**) (1 mmol), a phenol derivative (1.1 mmol) and DMAP (0.2 mmol) in CH_2Cl_2 (10 ml), EDC (1.1 mmol) was added, and stirred at room temperature overnight (DCC was used instead of EDC for compounds **18** and **19**). The reaction mixture was diluted with CH_2Cl_2 and washed with 0.5 N HCl (3 × 20 ml), 1% NaOH solution (3 × 20 ml) and brine. After drying over Na_2SO_4, solvent was evaporated off under reduced pressure and the crude product was purified by flash chromatography.

*4.3.1. 3-Chloro-6-(5-(4-methylphenyl)-3-{3-oxo-3-[4-(tetrahydrofuran-2-ylmethyl)piperazin-1-yl]propyl}-1H-pyrazol-1-yl)pyridazine (**9**)*

Prepared from **4a** and 1-(tetrahydrofuran-2-yl)methyl)piperazine using the procedure 1. Flash chromatography CH_2Cl_2:MeOH (95:5); yield 68%; Mp 128–129 °C; IR (ATR): 1649 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.47 (m, 1H), 1.87 (m, 2H), 1.99 (m, 1H), 2.35 (s, 3H), 2.38–2.55 (m, 6H), 2.78 (t, 2H, J = 7.6 Hz), 3.08 (t, 2H, J = 7.6 Hz), 3.53 (t, 2H), 3.64–3.89 (m, 4H), 4.02 (m, 1H), 6.38 (s, 1H), 7.17 (m, 4H), 7.53 (d, 1H, J = 9.2 Hz), 7.83 (d, 1H, J = 9.6 Hz); HRMS (m/z): $[M + H]^+$calcd for $C_{26}H_{32}ClN_6O_2$, 495.2275; found, 495.2275.

*4.3.2. 3-[1-(6-Chloropyridazin-3-yl)-5-(4-methylphenyl)-1H-pyrazol-3-yl]-N-(6-morpholin-4-ylpyridin-3-yl)propanamide (**10**)*

Prepared from **4a** and 6-(morpholin-4-yl)pyridin-3-amine using the procedure 1. Flash chromatography CH_2Cl_2:MeOH (95:5), after recrystallized from ethanol; yield 26.7%; Mp 242–243 °C; IR (ATR): 1686 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.35 (s, 3H), 2.83 (t, 2H, J = 6.8 Hz), 3.15 (t, 2H, J = 6.8 Hz), 3.43 (t, 4H, J = 4.6 Hz), 3.81 (t, 4H, J = 4.6 Hz), 6.38 (s, 1H), 6.61 (d, 1H, J = 8.8 Hz), 7.15 (s, 4H), 7.51 (d, 1H, J = 8.8 Hz), 7.72 (m, 2H), 7.90 (d, 1H, J = 8.8 Hz), 8.13 (s, 1H); HRMS (m/z): $[M + H]^+$calcd for $C_{26}H_{27}ClN_7O_2$, 504.1915; found, 504.1892.

*4.3.3. 3-Chloro-6-[3-{3-[4-(ethylsulfonyl)piperazin-1-yl]-3-oxopropyl}-5-(4-methylphenyl)-1H-pyrazol-1-yl]pyridazine (**11**)*

Prepared from **4a** and 1-(ethylsulfonyl)piperazine using the procedure 1. Flash chromatography CH_2Cl_2:MeOH (97.5:2.5); yield 40.7%; Mp 161–162 °C; IR (ATR): 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.36 (t, 3H, J = 7.4 Hz), 2.36 (s, 3H), 2.81 (t, 2H, J = 7.6 Hz), 2.94 (q, 2H, J = 7.4 Hz), 3.10 (t, 2H, J = 7.4 Hz), 3.27 (t, 4H), 3.60 (t, 2H), 3.74 (t, 2H), 6.39 (s, 1H), 7.15 (s, 4H), 7.53 (d, 1H, J = 8.8 Hz), 7.75 (d, 1H, J = 9.2 Hz); ^{13}C NMR ($CDCl_3$): δ 7.97, 21.53, 23.99, 32.22, 41.92, 44.45, 45.63, 45.92, 46.09, 110.16, 124.73, 127.60, 128.83, 129.40, 130.00, 139.01, 146.01, 154.94, 155.18, 155.37, 170.75. HRMS (m/z): $[M + H]^+$calcd for $C_{23}H_{28}ClN_6O_3S$, 503.1632; found, 503.1631.

*4.3.4. 4-Isopropylphenyl 3-[1-(6-chloropyridazin-3-yl)-5-(4-methylphenyl)-1H-pyrazol-3-yl]propanoate (**12**)*

Prepared from **4a** and 4-isopropylphenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH (99:1); yield 70.6%; Mp 160–160.7 °C; IR (ATR): 1750 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.23 (d, 6H, J = 6.8 Hz), 2.36 (s, 3H), 2.90 (m, 1H), 3.03 (t, 2H, J = 7.2 Hz), 3.17 (t, 2H, J = 7.4 Hz), 6.41 (s, 1H), 6.99 (m, 2H), 7.14–7.22 (m, 6H), 7.54 (d, 1H, J = 9.6 Hz), 7.90 (d, 1H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 21.56, 23.83, 24.26, 33.56, 33.83, 110.12, 121.37, 124.49, 127.56, 127.87, 128.93, 129.28, 130.10, 138.87, 146.16, 146.60, 148.81, 154.49, 154.78, 155.45, 171.77. HRMS (m/z): $[M + Na]^+$calcd for $C_{26}H_{25}ClN_4O_2Na$, 483.1564; found, 483.1552.

*4.3.5. 4-[4-(Trifluoromethyl)phenoxy]phenyl 3-[1-(6-chloropyridazin-3-yl)-5-(4-methylphenyl)-1H-pyrazol-3-yl] propanoate (**13**)*

Prepared from **4a** and 4-[4-(trifluoromethyl)phenoxy]phenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH (97:3); yield 49%; Mp 142.3–142.3 °C; IR (ATR): 1758 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H), 3.06 (t, 2H, J = 7.6 Hz), 3.19 (t, 2H, J = 7.2 Hz), 6.42 (s, 1H), 7.03–7.21 (m, 10H), 7.53–7.58 (m, 3H), 7.88 (d, 1H, J = 9.2 Hz); HRMS (m/z): $[M + H]^+$calcd for $C_{30}H_{23}ClF_3N_4O_3$, 579.1411; found, 579.1439.

*4.3.6. 4-(Trifluoromethoxy)phenyl 3-[1-(6-chloropyridazin-3-yl)-5-(4-methylphenyl)-1H-pyrazol-3-yl]propanoate (**14**)*

Prepared from **4a** and 4-(trifluoromethoxy)phenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH (96:4); yield 45%; Mp 131.3–132.3 °C; IR (ATR): 1758 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H), 3.06 (t, 2H, J = 7.0 Hz), 3.18 (t, 2H, J = 7.4 Hz), 6.41 (s, 1H), 7.10–7.20 (m, 8H), 7.54 (d, 1H, J = 9.2 Hz), 7.86 (d, 1H, J = 8.8); HRMS (m/z): $[M + H]^+$calcd for $C_{24}H_{19}ClF_3N_4O_3$,503.1098; found, 503.1102.

*4.3.7. 3-[3-{3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-(4-fluorophenyl)-1H-pyrazol-1-yl]-6-chloropyridazine (**15**)*

Prepared from **4b** and 1-(4-*t*-butylbenzyl)piperazine using the procedure 1. Flash chromatography CH_2Cl_2:MeOH (95:5); yield 56%; Mp 158.5–159.5 °C; IR (ATR): 1636 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.32 (s, 9H), 2.42 (m, 4H), 2.77 (t, 2H, J = 7.6 Hz), 3.08 (t, 2H, J = 7.6 Hz), 3.47 (s,2H), 3.50 (t, 2H), 3.65 (t, 2H), 6.38 (s, 1H), 7.04 (dd, 2H, J = 8.4 and 8.8 Hz), 7.22 (d, 2H, J = 8.4 Hz), 7.29 (dd, 2H, J = 8.8 Hz and 5.2 Hz), 7.34 (d, 2H, J = 8.4 Hz), 7.56 (d, 1H, J = 8.8 Hz), 7.95 (d, 1H, J = 9.2 Hz); ^{13}C NMR ($CDCl_3$): δ 24.02, 31.58, 32.27, 34.70, 41.95, 45.70, 52.99, 53.35, 62.76, 110.89, 115.56 ($^2J_{CF}$ = 21 Hz), 124.25, 125.43, 127.10 ($^4J_{CF}$ = 3 Hz), 129.04, 130.19, 130.96 ($^3J_{CF}$ = 8.4 Hz), 134.66, 144.87, 150.44, 154.76, 155.31, 155.63, 163.04 (J_{CF} = 247 Hz), 170.39. HRMS (m/z): $[M + H]^+$calcd for $C_{31}H_{35}ClFN_6O$, 561.2545; found, 561.2518.

*4.3.8. 3-Chloro-6-(5-(4-fluorophenyl)-3-{3-oxo-3-[4-(tetrahydrofuran-2-ylcarbonyl)piperazin-1-yl]propyl}-1H-pyrazol-1-yl)pyridazine (**16**)*

Prepared from **4b** and 1-((tetrahydrofuran-2-yl)carbonyl) piperazine using the procedure 1. Flash chromatography CH_2Cl_2:MeOH (95:5); yield 60.7%; Mp 162–164 °C; IR (ATR): 1636 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.90–2.34 (m, 4H), 2.82 (t, 2H, J = 7.6 Hz), 3.10 (t, 2H, J = 7.6 Hz), 3.42–3.93 (m, 10H), 4.59 (m, 1H), 6.39 (s, 1H), 7.04 (m, 2H), 7.29 (m, 2H), 7.57 (d, 1H, J = 8.8 Hz), 7.92 (m, 1H); HRMS (m/z): $[M + H]^+$calcd for $C_{25}H_{27}ClFN_6O_3$, 513.1817; found, 513.1807.

*4.3.9. 3-[1-(6-Chloropyridazin-3-yl)-5-(4-fluorophenyl)-1H-pyrazol-3-yl]-N-(4-methoxyphenyl)propanamide (**17**)*

Prepared from **4b** and 4-methoxyaniline using the procedure 1. Recrystallized from methanol; yield 47%; Mp 149–150 °C; IR (ATR): 1650 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.80 (t, 2H, J = 7.0 Hz), 3.15 (t, 2H, J = 7.4 Hz), 3.78 (s, 3H), 6.38 (s, 1H), 6.84 (m, 2H), 7.03 (m, 2H), 7.27 (m, 2H), 7.40 (m, 2H), 7.53 (d, 1H, J = 8.8 Hz), 7.59 (s, 1H), 7.86 (d, 1H, J = 9.2 Hz); HRMS (m/z): $[M + Na]^+$calcd for $C_{23}H_{19}ClFN_5O_2Na$, 474.1109; found, 474.1094.

*4.3.10. 4-tert-Butylphenyl 3-[1-(6-chloropyridazin-3-yl)-5-(4-fluorophenyl)-1H-pyrazol-3-yl]propanoate (**18**)*

Prepared from **4b** and 4-*t*-butylphenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH (95:5); yield 52.6%; Mp 168–169.5 °C; IR (ATR): 1752 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H),

3.04 (t, 2H, $J = 7$ Hz), 3.17 (t, 2H, $J = 7.4$ Hz), 6.41 (s, 1H), 6.99 (d, 2H, $J = 8.4$ Hz), 7.05 (m, 2H), 7.32 (m, 2H), 7.37 (d, 2H, $J = 8.4$ Hz), 7.57 (d, 1H, $J = 9.2$ Hz), 8.01 (d, 1H, $J = 9.6$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{26}H_{25}ClFN_4O_2$, 479.1650; found, 479.1661.

*4.3.11. 4-(3-Thienyl)phenyl 3-[1-(6-chloropyridazin-3-yl)-5-(4-fluorophenyl)-1H-pyrazol-3-yl]propanoate (**19**)*

Prepared from **4b** and 4-(thiophen-3-yl)phenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH (95:5); yield 41.5%; Mp 187–188 °C; IR (ATR): 1743 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.07 (t, 2H, $J = 7.2$ Hz), 3.19 (t, 2H, $J = 7.2$ Hz), 6.42 (s, 1H), 7.05 (dd, 2H, $J = 8.8$ Hz and 8.4 Hz), 7.11 (d, 2H, $J = 8.4$ Hz), 7.30–7.41 (m, 5H), 7.56–7.59 (m, 3H), 8.00 (d, 1H, $J = 9.2$ Hz); HRMS (*m*/*z*): $[M + Na]^+$calcd for $C_{26}H_{18}ClFN_4O_2SNa$, 527.0721; found, 527.0720.

*4.3.12. 4-(Heptyloxy)phenyl 3-[1-(6-chloropyridazin-3-yl)-5-(4-fluorophenyl)-1H-pyrazol-3-yl]propanoate (**20**)*

Prepared from **4b** and 4-heptyloxyphenol using the procedure 2. Recrystallized from ethyl acetate-hexane; yield 64.4%; Mp 136–137 °C; IR (ATR): 1746 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (t, 3H, $J = 6.6$ Hz), 1.30–1.46 (m, 8H), 1.76 (m, 2H), 3.02 (t, 2H, $J = 7.2$ Hz), 3.17 (t, 2H, $J = 7.2$ Hz), 3.92 (t, 2H, $J = 6.6$ Hz), 6.40 (s, 1H), 6.86 (d, 2H, $J = 8.8$ Hz), 6.97 (d, 2H, $J = 8.8$ Hz), 7.05 (dd, 2H, $J = 8.8$ Hz and 8.4 Hz), 7.32 (dd, 2H, $J = 8.4$ Hz and 5.2 Hz), 7.57 (d, 1H, $J = 8.8$ Hz), 8.00 (d, 1H, $J = 9.6$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{29}H_{31}ClFN_4O_3$, 537.2069; found, 537.2048.

*4.3.13. 4-(1H-Imidazol-1-yl)phenyl 3-[1-(6-chloropyridazin-3-yl)-5-(4-fluorophenyl)-1H-pyrazol-3-yl]propanoate (**21**)*

Prepared from **4b** and 4-(1*H*-imidazol-1-yl)phenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH (95:5); yield 60.9%; Mp 112–113 °C; IR (ATR): 1752 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.09 (t, 2H, $J = 7.2$ Hz), 3.20 (t, 2H, $J = 7.2$ Hz), 6.42 (s, 1H), 7.06 (dd, 2H, $J = 8.8$ Hz and 8.4 Hz), 7.20–7.23 (m, 3H), 7.25 (m, 1H), 7.32 (dd, 2H, $J = 8.8$ Hz and 5.2 Hz), 7.39 (m, 2H), 7.58 (d, 1H, $J = 9.2$ Hz), 7.81 (s, 1H), 7.98 (d,1H, $J = 8.8$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{25}H_{19}ClFN_6O_2$, 489.1242; found, 489.1223.

*4.3.14. 1-(3-{1-(6-Chloropyridazin-3-yl)-5-[4-(trifluoromethyl) phenyl]-1H-pyrazol-3-yl}propanoyl)piperidine-4-carboxamide (**22**)*

Prepared from **4c** and piperidin-4-carboxamide using the procedure 1. Recrystallized from methanol; Yield 51%; Mp 156–157 °C; IR (ATR): 1649, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.66 (m, 2H), 1.90 (m, 2H), 2.41 (m, 1H), 2.67–3.16 (m, 6H), 3.98 (d, 1H), 4.65 (m,1H), 5.50 (m, 2H), 6.46 (s, 1H), 7.43 (d, 2H, $J = 8.0$ Hz), 7.61 (d, 2H, $J = 8.0$ Hz), 7.64 (d, 1H, $J = 8.8$ Hz), 8.05 (d, 1H, $J = 8.8$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{23}H_{23}ClF_3N_6O_2$, 507.1523; found, 507.1518.

*4.3.15. Ethyl 1-(3-{1-(6-chloropyridazin-3-yl)-5-[4-(trifluoromethyl)phenyl]-1H-pyrazol-3-yl}propanoyl)piperidine-4-carboxylate (**23**)*

Prepared from **4c** and ethyl piperidin-4-carboxylate using the procedure 1. Flash chromatography CH_2Cl_2:MeOH (98:2); yield 54.3%; Mp 121–122 °C; IR (ATR): 1733, 1652 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.26 (t, 3H, $J = 7.0$ Hz), 1.66 (m, 2H), 1.94 (m, 2H), 2.54 (m, 1H), 2.75–3.17 (m, 6H), 3.90 (m, 1H), 4.15 (q, 2H, $J = 7.0$ Hz), 4.47 (m, 1H), 6.46 (s, 1H), 7.44 (d, 2H, $J = 7.6$ Hz), 7.61 (m, 3H), 8.05 (d, 1H, $J = 8.8$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{25}H_{26}ClF_3N_5O_3$, 536.1676; found, 536.1674.

*4.3.16. 3-Chloro-6-{3-{3-[4-(2-furoyl)piperazin-1-yl]-3-oxopropyl}-5-[4-(trifluoromethyl) phenyl]-1H-pyrazol-1-yl} pyridazine (**24**)*

Prepared from **4c** and 1-((furan-2-yl)carbonyl)piperazine using the procedure 1. Flash chromatography CH_2Cl_2:MeOH (95:5); yield 44%; Mp 151–152 °C; IR (ATR): 1619 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.85 (t, 2H, $J = 7.4$ Hz), 3.13 (t, 2H, $J = 7.4$ Hz), 3.60–3.82 (m, 8H), 6.46 (s, 1H), 6.51 (dd, 1H, $J = 3.6$ Hz and 2 Hz), 7.07 (d, 1H, $J = 3.2$ Hz), 7.44 (d, 2H, $J = 8.0$ Hz), 7.50 (s, 1H), 7.60 (m, 3H), 8.02 (d, 1H, $J = 8.8$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{26}H_{23}ClF_3N_6O_3$, 559.1472; found, 559.1498.

*4.3.17. 4-Cyclopentylphenyl 3-{1-(6-chloropyridazin-3-yl)-5-[4-(trifluoromethyl)phenyl]-1H-pyrazol-3-yl}propanoate (**25**)*

Prepared from **4c** and 4-(cyclopentyl)phenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH (80:20); yield 43.5%; Mp 157–158 °C; IR (ATR): 1759 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.52–2.06 (m, 9H), 3.04 (t, 2H, $J = 7.2$ Hz), 3.18 (t, 2H, $J = 7.2$ Hz), 6.47 (s, 1H), 6.97 (d, 2H, $J = 8.4$ Hz), 7.22 (d, 2H, $J = 8.4$ Hz), 7.45 (d, 2H, $J = 8.4$ Hz), 7.61 (m, 3H), 8.08 (d, 1H, $J = 9.2$ Hz); HRMS (*m*/*z*): $[M + Na]^+$calcd for $C_{28}H_{24}ClF_3N_4O_2Na$, 563.1438; found, 563.1432.

*4.3.18. 4-(1H-Pyrrol-1-yl)phenyl 3-{1-(6-chloropyridazin-3-yl)-5-[4-(trifluoromethyl)phenyl]-1H-pyrazol-3-yl}propanoate (**26**)*

Prepared from **4c** and 4-(1*H*-pyrrol-1-yl)phenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH (96:4); yield 39%; Mp 131–132 °C; IR (ATR): 1749 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.08 (t, 2H, $J = 7$ Hz), 3.20 (t, 2H, $J = 7$ Hz), 6.35 (dd, 2H, $J = 2.4$ and 2.0 Hz), 6.48 (s, 1H), 7.04 (dd, 2H, $J = 2.4$ and 2.0 Hz), 7.14 (d, 2H, $J = 8.8$ Hz), 7.38 (d, 2H, $J = 8.8$ Hz), 7.46 (d, 2H, $J = 8.4$ Hz), 7.61 (d, 1H, $J = 9.2$ Hz), 7.62 (d, 2H, $J = 8.4$ Hz), 8.07 (d, 1H, $J = 9.2$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{27}H_{20}ClF_3N_5O_2$, 538.1258; found, 538.1253.

*4.3.19. 3-[3-{3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-(4-methylphenyl)-1H-pyrazol-1-yl]-6-chloropyridazine (**27**)*

To a mixture of **4a** (1 mmol), 1-(4-*t*-butylbenzyl)piperazine (1.1 mmol) and DMAP (0.2 mmol) in CH_2Cl_2 (10 ml), EDC (1.1 mmol) was added and stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with 1N HCl (3 × 25 ml), 5% $NaHCO_3$ solution (3 × 25 ml) and water. After drying over Na_2SO_4 the solvent was evaporated off under reduced pressure to dryness. The crude product was purified by flash chromatography using CH_2Cl_2:MeOH (95:5); Yield 67.7%; Mp 148.5–151.5 °C; IR (ATR): 1634 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.32 (s, 9H), 2.35 (s, 3H), 2.40 (m, 4H), 2.77 (t, 2H), 3.08 (t, 2H), 3.47 (s, 2H), 3.49 (t, 2H), 3.65 (t, 2H), 6.38 (s, 1H), 7.14 (d, 2H, $J = 8.0$ Hz), 7.18 (d, 2H, $J = 8.0$ Hz), 7.22 (d, 2H, $J = 8.4$ Hz), 7.34 (d, 2H, $J = 8.4$ Hz), 7.53 (d, 1H, $J = 8.8$ Hz), 7.82 (d, 1H, $J = 9.2$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{32}H_{38}ClN_6O$, 557.2796; found, 557.2799.

The same procedure was applied to convert **4a,c** to **27, 28**; **5a–c** to **29–31**; **6a–c** to **32–34**; **7a–c** to **35–37** and **8a–c** to **38–40**, respectively.

*4.3.20. 3-{3-[3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-[4-(trifluoromethyl) phenyl]-1H-pyrazol-1-yl}-6-chloropyridazine (**28**)*

Prepared from **4c** and purified by flash chromatography using CH_2Cl_2:MeOH (95:5); yield 68%; Mp 155.5–156 °C; IR (ATR): 1651 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.43 (s, 4H), 2.78 (t, 2H, $J = 7.6$ Hz), 3.09 (t, 2H, $J = 7.6$ Hz), 3.48 (m, 4H), 3.66 (m, 2H), 6.45 (s, 1H), 7.23 (d, 2H, $J = 8.0$ Hz), 7.34 (d, 2H, $J = 8.0$ Hz), 7.44 (d, 2H, $J = 8.0$ Hz), 7.59 (d, 1H, $J = 8.8$), 7.61 (2H, d, $J = 8.0$ Hz), 8.03 (d, 1H, $J = 9.6$ Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{32}H_{35}ClF_3N_6O$, 611.2513; found, 611.2510.

*4.3.21. 1-(4-tert-Butylbenzyl)-4-{3-[1-(4-chlorophenyl)-5-(4-methylphenyl)-1H-pyrazol-3-yl]propanoyl}piperazine (**29**)*

Prepared from **5a** and purified by flash chromatography CH_2Cl_2:MeOH (95:5); yield 78%; Mp 246–247 °C; IR (ATR):

1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.32 (s, 9H), 2.35 (s, 3H), 2.40 (m, 4H), 2.77 (m, 2H), 3.06 (m, 2H), 3.45 (s, 2H), 3.56 (m, 2H), 3.64 (m, 2H), 6.32 (s, 1H), 7.07–7.13 (m, 4H), 7.19–7.23 (m, 4H), 7.28 (m, 2H), 7.34 (m, 2H); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{34}H_{40}ClN_4O$, 555.2891; found, 555.2871.

*4.3.22. 1-(4-tert-Butylbenzyl)-4-{3-[1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrazol-3-yl]propanoyl}piperazine (**30**)*

Prepared from **5b** and purified by flash chromatography CH_2Cl_2:MeOH (95:5); yield 64%; Mp 245–246 °C; IR (ATR): 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.39 (m, 4H), 2.77 (t, 2H, *J* = 7.6), 3.06 (t, 2H, *J* = 7.6), 3.46 (s, 2H), 3.50 (m, 2H), 3.65 (m, 2H), 6.34 (s, 1H), 7.01 (m, 2H), 7.16–7.23 (m, 6H), 7.30 (m, 2H), 7.34 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 24.08, 31.60, 32.74, 34.71, 41.93, 45.75, 53.02, 53.40, 62.78, 107.85, 115.93 ($^2J_{CF}$ = 22 Hz), 125.43, 126.40, 126.73 ($^4J_{CF}$ = 3.8 Hz), 129.03, 129.32, 130.69 ($^3J_{CF}$ = 7.6 Hz), 133.12, 134.71, 138.68, 142.85, 150.42, 153.38, 162.83 (J_{CF} = 247 Hz), 170.74. HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{33}H_{37}ClFN_4O$, 559.2640; found, 559.2660.

*4.3.23. 1-(4-tert-Butylbenzyl)-4-(3-{1-(4-chlorophenyl)-5-[4-(trifluoromethyl)phenyl]-1H-pyrazol-3-yl}propanoyl)piperazine (**31**)*

Prepared from **5c** and purified by flash chromatography CH_2Cl_2:MeOH (90:10); yield 65%; Mp 272–273 °C; IR (ATR): 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.40 (m, 4H), 2.78 (t, 2H, *J* = 7.6), 3.08 (t, 2H, *J* = 7.6), 3.46 (s, 2H), 3.50 (m, 2H), 3.65 (m, 2H), 6.43 (s, 1H), 7.18–7.23 (m, 4H), 7.30–7.35 (m, 6H), 7.57 (d, 2H, *J* = 8.0); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{34}H_{37}ClF_3N_4O$, 609.2608; found, 609.2621.

*4.3.24. 1-(4-tert-Butylbenzyl)-4-{3-[5-(4-methylphenyl)-1-phenyl-1H-pyrazol-3-yl]propanoyl}piperazine (**32**)*

Prepared from **6a** and purified by flash chromatography CH_2Cl_2:MeOH (80:20); yield 27%; Mp 223–225 °C; IR (ATR): 1642 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.30 (s, 9H), 2.34 (m, 3H), 2.40 (m, 4H), 2.79 (t, 2H, *J* = 7.6 Hz), 3.06 (t, 2H, *J* = 7.6 Hz), 3.45 (s, 2H), 3.51 (m, 2H), 3.63 (m, 2H), 6.32 (s, 1H), 7.00 (m, 2H), 7.10–7.37 (m, 11H); ^{13}C NMR ($CDCl_3$): δ 21.46, 24.00, 31.36, 31.52, 35.01, 38.63, 42.63, 51.10, 51.91, 60.91, 107.18, 125.50, 126.56, 127.61, 127.64, 128.67, 129.25, 129.41, 131.39, 138.49, 140.48, 144.19, 152.20, 153.86, 171.23. HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{34}H_{41}N_4O$, 521.3280; found, 521.3270.

*4.3.25. 1-(4-tert-Butylbenzyl)-4-{3-[5-(4-fluorophenyl)-1-phenyl-1H-pyrazol-3-yl]propanoyl}piperazine (**33**)*

Prepared from **6b** and purified by flash chromatography CH_2Cl_2:MeOH (97:3); yield 68%; Mp 224–225 °C; IR (ATR): 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.32 (s, 9H), 2.39 (m, 4H), 2.78 (t, 2H, *J* = 7.6 Hz), 3.08 (t, 2H, *J* = 7.6 Hz), 3.46 (s, 2H), 3.50 (m, 2H), 3.65 (m, 2H), 6.34 (s, 1H), 6.98 (m, 2H), 7.16–7.35 (m, 11H); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{33}H_{38}FN_4O$, 525.3030; found, 525.3041.

*4.3.26. 1-(4-tert-Butylbenzyl)-4-(3-{1-phenyl-5-[4-(trifluoromethyl)phenyl]-1H-pyrazol-3-yl}propanoyl)piperazine (**34**)*

Prepared from **6c** and purified by flash chromatography CH_2Cl_2:MeOH (90:10); yield 61%; Mp 264–265 °C; IR (ATR): 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.51 (m, 4H), 2.80 (m, 2H), 3.09 (m, 2H), 3.5–3.62 (m, 6H), 6.43 (s, 1H), 7.24–7.38 (m, 11H), 7.54 (d, 2H, *J* = 8.4 Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{34}H_{38}F_3N_4O$, 575.2998; found, 575.2973.

*4.3.27. 2-[3-{3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-(4-methylphenyl)-1H-pyrazol-1-yl]quinoline (**35**)*

Prepared from **7a** and purified by flash chromatography CH_2Cl_2:MeOH (95:5); yield 83.7%; Mp 166–167 °C; IR (ATR): 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.35 (s, 3H), 2.41 (m, 4H), 2.82 (m, 2H), 3.13 (m, 2H), 3.45 (s, 2H), 3.51 (m, 2H), 3.66 (m, 2H), 6.41 (s, 1H), 7.09 (d, 2H, *J* = 8.4 Hz), 7.19–7.22 (m, 4H), 7.32 (d, 2H, *J* = 8.0 Hz), 7.44 (d, 1H, *J* = 8.4 Hz), 7.51 (m, 1H), 7.67 (m, 1H), 7.80 (d, 1H, *J* = 7.2 Hz), 7.88 (d, 1H, *J* = 8.8 Hz), 8.11 (d, 1H, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$): δ 21.53, 24.31, 31.61, 32.89, 34.70, 41.91, 45.77, 53.03, 53.41, 62.76, 108.97, 117.65, 125.42, 126.78, 127.12, 127.59, 128.48, 128.98, 129.06, 129.08, 129.46, 130.20, 134.74, 138.31, 138.34, 145.06, 146.95, 150.37, 151.35, 154.19, 170.85. HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{37}H_{42}N_5O$, 572.3389; found, 572.3394.

*4.3.28. 2-[3-{3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-(4-fluorophenyl)-1H-pyrazol-1-yl]quinoline (**36**)*

Prepared from **7b** and purified by flash chromatography CH_2Cl_2:MeOH (95:5); yield 58.5%; Mp 223–224 °C; IR (ATR): 1642 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.40 (m, 4H), 2.81 (m, 2H), 3.13 (m, 2H), 3.45 (s, 2H), 3.51 (m, 2H), 3.66 (m, 2H), 6.40 (s, 1H), 7.00 (dd, 2H, *J* = 8.4 Hz and *J* = 8.8 Hz), 7.21 (d, 2H, *J* = 8.4 Hz), 7.28–7.34 (m, 4H), 7.51 (m, 1H), 7.63 (m, 2H), 7.73 (d, 1H, *J* = 8.0 Hz), 7.80 (d, 1H, *J* = 8.0 Hz), 8.17 (d, 1H, *J* = 9.2 Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{36}H_{39}FN_5O$, 576.3139; found, 576.3158.

*4.3.29. 2-{3-{3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-[4-(trifluoromethyl) phenyl]-1H-pyrazol-1-yl} quinoline (**37**)*

Prepared from **7c** and purified by flash chromatography CH_2Cl_2:MeOH (95:5); yield 24%; Mp 163–164 °C; IR (ATR): 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.41 (m, 4H), 2.82 (t, 2H, *J* = 7.6 Hz), 3.14 (t, 2H, *J* = 7.6 Hz), 3.46 (s, 2H), 3.51 (m, 2H), 3.67 (m, 2H), 6.47 (s, 1H), 7.21 (d, 2H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 8.4 Hz), 7.44 (d, 2H, *J* = 8.4 Hz), 7.51 (m, 1H), 7.57 (d, 2H, *J* = 8.0 Hz), 7.61–7.64 (m, 2H), 7.75 (d, 1H, *J* = 8.8 Hz), 7.80 (d, 1H, *J* = 8.0 Hz), 8.20 (d, 1H, *J* = 8.8 Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{37}H_{39}F_3N_5O$, 626.3107; found, 626.3129.

*4.3.30. 3-[3-{3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-(4-methylphenyl)-1H-pyrazol-1-yl]-6-methoxypyridazine (**38**)*

Prepared from **8a** and purified by flash chromatography CH_2Cl_2:MeOH (90:10); yield 48%; Mp 121–122 °C; IR (ATR): 1641 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.34 (s, 3H), 2.40 (m, 4H), 2.77 (m, 2H), 3.07 (t, 2H), 3.46 (s, 2H), 3.49 (m, 2H), 3.65 (m, 2H), 4.10 (s, 3H), 6.36 (s, 1H), 7.02 (d, 1H, *J* = 8.8 Hz) 7.11 (d, 2H, *J* = 8.0 Hz), 7.16 (d,2H, *J* = 8.0 Hz), 7.22 (d, 2H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 8.0 Hz), 7.59 (d,1H, *J* = 9.6 Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{33}H_{41}N_6O_2$,553.3291; found, 553.3271.

*4.3.31. 3-[3-{3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-(4-fluorophenyl)-1H-pyrazol-1-yl]-6-methoxypyridazine (**39**)*

Prepared from **8b** and purified by flash chromatography CH_2Cl_2:MeOH (95:5); yield 65%; Mp 151–152 °C; IR (ATR): 1648 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.40 (m, 4H), 2.77 (m, 2H), 3.07 (t, 2H), 3.47 (s, 2H), 3.50 (m, 2H), 3.65 (m, 2H), 4.09 (s, 3H), 6.36 (s, 1H), 7.01 (m, 2H), 7.06 (d, 1H, *J* = 8.8 Hz), 7.22 (d, 2H, *J* = 8.4 Hz), 7.26 (m, 2H), 7.34 (d, 2H, *J* = 8.4 Hz), 7.72 (d, 1H, *J* = 9.6 Hz); HRMS (*m*/*z*): $[M + H]^+$calcd for $C_{32}H_{38}FN_6O_2$, 557.3040; found, 557.3014.

*4.3.32. 3-{3-{3-[4-(4-tert-Butylbenzyl)piperazin-1-yl]-3-oxopropyl}-5-[4-(trifluoromethyl) phenyl]-1H-pyrazol-1-yl}-6-methoxypyridazine (**40**)*

Prepared from **8c** and purified by flash chromatography CH_2Cl_2:MeOH (95:5); yield 77%; Mp 124–125 °C; IR (ATR): 1646 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.32 (s, 9H), 2.42 (m, 4H), 2.78 (t,

2H,J = 7.6 Hz), 3.09 (t, 2H, J = 7.6 Hz), 3.47 (s, 2H) 3.50 (m, 2H), 3.66 (m, 2H), 4.09 (s, 3H), 6.45 (s, 1H), 7.10 (d, 1H, J = 9.2 Hz), 7.22 (d, 2H, J = 8.0), 7.34 (d, 2H, J = 8.4 Hz), 7.40 (d, 2H, J = 8.0 Hz), 7.58 (d, 2H, J = 8.8 Hz), 7.82 (d, 1H, J = 9.2 Hz). HRMS (m/z): $[M + H]^+$calcd for $C_{33}H_{38}F_3N_6O_2$, 607.3008; found, 607.3029.

4.3.33. Ethyl 1-{3-[5-(4-methylphenyl)-1-phenyl-1H-pyrazol-3-yl] propanoyl}piperidine-4-carboxylate (41)

Prepared from **6a** and ethyl piperidin-4-carboxylate using the same procedure for compound **27**; purified by flash chromatography CH_2Cl_2:MeOH (95:5); yield 38%; yellow oil; IR (ATR): 1639, 1726 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.25 (t, 3H, J = 7.2 Hz), 1.62 (m, 2H), 1.92 (m, 2H), 2.33 (s, 3H), 2.51 (m, 1H), 2.80 (m, 3H), 3.10 (m, 3H), 3.92 (m, 1H), 4.13 (q, 2H, J = 7.2 Hz), 4.45 (m, 1H), 6.34 (s, 1H), 7.09 (s, 5H), 7.27–7.34 (m, 4H); HRMS (m/z): $[M + H]^+$calcd for $C_{27}H_{32}N_3O_3$, 446.2444; found, 446.2448.

4.3.34. 4-Isopropylphenyl 3-[5-(4-methylphenyl)-1-phenyl-1H-pyrazol-3-yl]propanoate (42)

Prepared from **6a** and 4-isopropylphenol using the same procedure for compound **27**; Flash chromatography hexane-ethyl acetate (80:20); yield 86%; Mp 74–75 °C; IR (ATR): 1753 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.18 (d, 6H, J = 6.8 Hz), 2.29 (s, 3H), 2.89 (m, 1H), 3.01 (m, 4H), 6.52 (s, 1H), 6.99 (d, 2H, J = 8.4 Hz), 7.10 (d, 2H, J = 8.4 Hz), 7.16 (d, 2H, J = 8.0 Hz), 7.24 (m, 4H), 7.24 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 21.42, 23.77, 24.59, 33.44, 33.56, 107.28, 122.15, 125.64, 127.82, 127.98, 128.87, 129.66, 129.85, 138.43, 140.56, 143.86, 146.46, 149.19, 151.95, 172.08. HRMS (m/z): $[M + H]^+$ calculated for $C_{28}H_{29}N_2O_2$, 425.2229; found, 425.2209.

4.3.35. Ethyl 1-{3-[5-(4-methylphenyl)-1-quinolin-2-yl-1H-pyrazol-3-yl]propanoyl}piperidine-4-carboxylate (43)

Prepared from **7a** and ethyl piperidin-4-carboxylate using the same procedure for compound **27**; Flash chromatography CH_2Cl_2:MeOH (97:3); yield 78%; Mp 109–110 °C; IR (ATR): 1638, 1718 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.24 (t, 3H, J = 7.2 Hz), 1.65 (m, 2H), 1.92 (m, 2H), 2.35 (s, 3H), 2.51 (m, 1H), 2.83 (m, 3H), 3.12 (m, 3H), 3.90 (m, 1H), 4.13 (q, 2H, J = 7.2 Hz), 4.47 (m, 1H), 6.40 (s, 1H), 7.09 (d, 2H, J = 8.0 Hz), 7.19 (d, 2H, J = 8.0 Hz), 7.44 (d, 1H, J = 8.4 Hz), 7.52 (m, 1H), 7.67 (m, 1H), 7.79 (d, 1H, J = 8.0 Hz), 7.89 (d, 1H, J = 8.4 Hz), 8.11 (d, 1H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$): δ 14.40, 21.50, 24.29, 28.11, 28.69, 32.99, 41.27, 45.02, 60.79, 108.92, 117.65, 126.76, 127.10, 127.58, 128.46, 128.96, 129.06, 129.44, 130.18, 138.29, 138.34, 145.05, 146.94, 151.32, 154.19, 170.76, 174.42. HRMS (m/z): $[M + H]^+$calcd for $C_{30}H_{33}N_4O_3$, 497.2553; found, 497.2530.

4.3.36. 4-Isopropylphenyl 3-[5-(4-methylphenyl)-1-quinolin-2-yl-1H-pyrazol-3-yl]propanoate (44)

Prepared from **7a** and 4-isopropylphenol using the same procedure for compound **27**; Flash chromatography hexane-ethyl acetate (85:15); yield 92%; Mp 81–82 °C; IR (ATR): 1755 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.23 (d, 6H, J = 6.8 Hz), 2.36 (s, 3H), 2.90 (m, 1H), 3.06 (t, 2H J = 7.6 Hz), 3.23 (t, 2H, J = 7.6 Hz), 6.43 (s, 1H), 7.02 (m, 2H), 7.10 (m, 2H), 7.21 (m, 4H), 7.52 (m, 2H), 7.67 (m, 1H), 7.80 (d, 1H, J = 8.4 Hz), 7.86 (d, 1H, J = 8.0 Hz), 8.14 (d, 1H, J = 8.4 Hz); HRMS (m/z): $[M + H]^+$ calculated for $C_{31}H_{30}N_3O_2$, 476.2338; found, 476.2322.

4.4. Biological assays

BWA4C, Arachidonic acid, Ca^{2+}-ionophore A23187 and all other fine chemicals were from Sigma (Deisenhofen, Germany), unless stated otherwise. HPLC solvents were from Merck (Darmstadt, Germany).

4.4.1. Cell Preparation

Human neutrophils were freshly isolated from leukocyte concentrates obtained at the Blood Center of the University Hospital Tuebingen (Germany). In brief, venous blood was taken from healthy adult donors and leukocyte concentrates were prepared by centrifugation at 4000g for 20 min at RT. Neutrophils were immediately isolated by dextran sedimentation, centrifugation on Nycoprepcushions (PAA Laboratories, Linz, Austria), and hypotonic lysis of erythrocytes as described [29]. Cells were finally resuspended in phosphate-buffered saline pH 7.4 (PBS) containing 1 mg/mL glucose and 1 mM $CaCl_2$ (PGC buffer) (purity >96–97%).

4.4.2. Expression of human recombinant 5-LO in E. coli and preparation of high speed supernatants

E. coli MV1190 was transformed with pT3-5LO plasmid and recombinant 5-LO protein was expressed at 30 °C as described [29]. Cells were lysed by incubation in 50 mM triethanolamine/HCl pH 8.0, 5 mM EDTA, soybean trypsin inhibitor (60 μg/mL), 1 mM phenylmethylsulfonylfluoride (PMSF), and lysozyme (1 mg/mL), homogenized by sonication (3 × 15 s), and centrifuged at 40,000 g for 20 min at 4 °C. The resulting 40,000 g supernatant was immediately used for 5-LO activity assays.

4.4.3. Determination of 5-LO, 12-LO and 15-LO product formation in intact cells

For assays of intact cells, 5 × 10^6 freshly isolated neutrophils were resuspended in 1 mL PGC buffer. After preincubation with the test compounds for 15 min at RT, 5-LO product formation was started by addition of 2.5 μM ionophore A23187 plus 20 μM AA. After 10 min at 37 °C, the reaction was stopped with 1 mL of methanol and 30 μL of 1 N HCl, 200 ng of prostaglandin B_1, and 500 μL of PBS were added. Formed 5-LO metabolites were extracted and analyzed by HPLC as described [30]. 5-LO product formation is expressed as ng of 5-LO products per 10^6 cells, which includes LTB_4 and its all-trans isomers, and 5(*S*)-hydro(pero)xy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5(S)-H(p)ETE). Cysteinyl LTs C_4, D_4, and E_4 were not detected, and oxidation products of LTB_4 were not determined. 15-H(P)ETE was analyzed as product of 15-LO and 12-H(P)ETE as product of 12-LO.

4.4.4. Determination of 5-LO product formation in cell-free systems

For determination of the activity of 5-LO in 40,000 g supernatants, aliquots of the supernatants (approximately 20 μL, corresponding to 4 mL *E. coli* cell culture) were added to 1 mL of a 5-LO reaction mix (PBS, pH 7.4 containing 1 mM EDTA). Samples were pre-incubated with the test compounds for 10 min at 4 °C, samples were prewarmed for 30 s at 37 °C, and 2 mM $CaCl_2$ and 20 μM AA were added to start 5-LO product formation. The reaction was stopped after 10 min at 37 °C by addition of 1 mL ofice-cold methanol, and the formed metabolites were analyzed as described for intact cells.

4.4.5. Induction of mPGES-1 expression in A549 cells, isolation of Microsomes, and determination of mPGES-1 inhibition

Preparation of A549 cells and determination of mPGES-1 activity was performed as described previously [23]. In brief, A549 cells were incubated for 16 h at 37 °C and 5% CO_2. Subsequently, the culture medium was replaced by fresh DMEM/High glucose (4.5 g/l) medium containing fetal calf serum (2%, v/v); IL-1β (1 ng/ml) was added, and cells were incubated for another 72 h. Thereafter, cells were detached with trypsin/EDTA, washed with PBS, and frozen in liquid nitrogen. Ice-cold homogenisation buffer (0.1 M potassium phosphate buffer pH 7.4, 1 mM phenylmethanesulfonylfluoride, 60 μg/ml soybean trypsin inhibitor, 1 μg/ml leupeptin, 2.5 mM glutathione, and 250 mM sucrose) was

added, and after 15 min, cells were resuspended and sonicated on ice (3 × 20 s). The homogenate was subjected to differential centrifugation at 10,000 × g for 10 min and 174,000 × g for 1 h at 4 °C. The pellet (microsomal fraction) was resuspended in 1 ml homogenization buffer, and the total protein concentration was determined by Coomassie protein assay. Microsomal membranes were diluted in potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione to give a final concentration of 50 ìg/ml. Test compounds or vehicle (1% DMSO) were added, and after 15 min at 4 °C, the reaction (100 μl total volume) was initiated by addition of PGH_2 (20 μM, final concentration). After 1 min at 4 °C, the reaction was terminated using stop solution (100 μl; 40 mM $FeCl_2$, 80 mM citric acid, and 10 μM of 11β-PGE_2). PGE_2 was separated by solid phase extraction on reversed phase (RP)-C18 material as previously described [23]. 11β-PGE_2 was used as internal standard to quantify PGE_2 product formation by integration of the area under the peaks.

4.4.6. COX-1 activity in platelets

Platelets were freshly isolated from leukocyte concentrates obtained at the Blood Center, University Hospital, Tuebingen, Germany. In brief, venous blood was subjected to centrifugation at 4000 × g/20 min/20 °C for preparation of leukocyte concentrates. Platelet-rich-plasma was prepared by dextran sedimentation and centrifugation on Lymphoprep cushions (PAA Laboratories, Linz, Austria). For isolation of platelets, platelet-rich-plasma was obtained from the supernatants, mixed with phosphate-buffered saline (PBS), pH 5.9 (3:2 v/v) and centrifuged (2100 × g, 15 min, RT). The pelleted platelets were resuspended in PBS, pH 5.9/0.9% NaCl (1:1, v/v) and centrifuged again. To determine 12-HHT formation, platelets (10^8/ml PBS containing 1 mM $CaCl_2$) were pre-incubated with the test compounds for 4 min at 4 °C for 4 min followed by 1 min at 37 °C. After addition of 5 μM AA and further incubation for 5 min at 37 °C, reaction was stopped by addition of 1 mL methanol and 30 μL of 1 N HCl, 200 ng of prostaglandin B_1, and 500 μL of PBS were added.The COX-1 product (12-HHT) was extracted and then analyzed by HPLC as described [31].

4.4.7. Determination of COX-2 inhibition in intact A 549 cells

Cells (2 × 10^6) were plated in 175 cm^2 flasks and incubated for 16 h at 37 °C and 5% CO_2. Thereafter, medium was replaced by fresh DMEM/High glucose (4.5 g/l) medium containing FCS (2%, v/v), and the cells were stimulated with interleukin-1β (1 ng/ml) for 72 h. After trypsination, the cells were washed twice with PBS. For determination of the COX product 6-keto $PGF_{1\alpha}$, 10^6 cells in 1 ml PBS containing 1 mM $CaCl_2$ were pre-incubated with the test compounds for 10 min at 37 °C, and 6-keto-$PGF_{1\alpha}$ formation was initiated by addition of 3 μM AA. After 15 min at 37 °C, the reaction was stopped by cooling on ice. Cells were centrifuged (800 × g, 5 min, 4 °C) and the amount of released 6-keto $PGF_{1\alpha}$ in the supernatant was quantified using a 6-keto $PGF_{1\alpha}$ High Sensitivity EIA Kit (Assay Designs, Ann Arbor, MI) according to the manufacturer's protocol.

4.4.8. Statistics

Data are expressed as mean ± SE. IC_{50} values, obtained from measurements at 4–5 different concentrations of the compounds, are approximations determined by graphical analysis (linear interpolation between the points between 50% activity). The program GraphpadInstat (Graphpad Software Inc., San Diego, CA) was used for statistical comparisons. Statistical evaluation of the data was performed by one-way ANOVAs for independent or correlated samples followed by Tukey HSD posthoc tests. A P value of <0.05 (*) was considered significant.

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