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Discovery of trisubstituted pyrazolines as a novel scaffold for the development of selective phosphodiesterase 5 inhibitors

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ABSTRACT

Celecoxib, is a selective cyclooxygenase-2 (COX2) inhibitor with a 1,5-diaryl pyrazole scaffold. Celecoxib has a better safety profile compared to other COX2 inhibitors having side effects of systemic hypertension and thromboembolic complications. This may be partly attributed to an off-target activity involving phosphodiesterase 5 (PDE5) inhibition and the potentiation of NO/cGMP signalling allowing coronary vasodilation and aortic relaxation. Inspired by the structure of celecoxib, we synthesized a chemically diverse series of compounds containing a 1,3,5-trisubstituted pyrazoline scaffold to improve PDE5 inhibitory potency, while eliminating COX2 inhibitory activity. SAR studies for PDE5 inhibition revealed an essential role for a carboxylic acid functionality at the 1-phenyl and the importance of the non-planar pyrazoline core over the planar pyrazole with the 5-phenyl moiety tolerating a range of substituents. These modifications led to new PDE5 inhibitors with approximately 20-fold improved potency to inhibit PDE5 and no COX-2 inhibitory activity compared with celecoxib. PDE isozyme profiling of compound **11** revealed a favorable selectivity profile. These results suggest that trisubstituted pyrazolines provide a promising scaffold for further chemical optimization to identify novel PDE5 inhibitors with potential for less side effects compared with available PDE5 inhibitors used for the treatment of penile erectile dysfunction and pulmonary hypertension.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed drugs all over the world due to their anti-inflammatory, antipyretic, and analgesic effects which are exerted through inhibiting cyclooxygenase (COX), isozymes COX1 and COX2 [1]. Long-term use of conventional NSAIDs, that non-selectively inhibit COX1 and COX2, causes gastric and renal toxicities that is attributed to COX1 inhibition and the suppression of physiologically important prostaglandins. The identification and characterization of an inducible COX2 isozyme in the 1990s that is localized primarily in inflamed tissues was the start of a race to develop safer NSAIDs that selectivity target COX2, collectively known as coxibs. Coxibs have reduced gastric and renal toxicity compared to classical NSAIDs [2–4], but clinical trials involving large numbers of subjects revealed an increased risk of systemic hypertension and thromboembolic complications, including myocardial infarction, among patients receiving the COX2 inhibitor rofecoxib (Vioxx®) which resulted in a number of coxibs being pulled from the market. This unexpected toxicity was attributed to an imbalance between thromboxane and prostacyclin levels whereby only prostacyclin synthesis is inhibited by coxibs leading to unopposed vasoconstriction [5]. Among the coxibs, celecoxib (A, Fig. 1) (Celebrex®) was an exception as it showed lower cardiovascular risk and remains on the market [6]. As previously reported, the improved cardiovascular safety profile of celecoxib may be attributed to a potentiating effect on the NO/cGMP signalling pathway through an off-target effect involving the inhibition of phosphodiesterase 5 (PDE5) [7]. Thus, the PDE5 inhibitory activity of celecoxib may compensate for a decrease of prostacyclin-dependent cAMP generation by concomitantly increasing cGMP levels, which is supported by evidence that PDE5

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inhibitors have cardioprotective properties [8]. In addition, several other NSAIDs, most notably, sulindac, have been reported to inhibit PDE5 [9], which also has cardioprotective properties [10]. However, none of the other coxibs inhibit PDE5, which suggests a specific property of celecoxib that might be optimized chemically to develop novel PDE5 inhibitors useful for the treatment of erectile dysfunction and pulmonary arterial hypertension but do not have side effects associated with the non-selective inhibition of other PDE isozymes [7].

Cyclic nucleotide PDEs are a large superfamily of phosphohydrolases which degrade the 3′-cyclic phosphate bond in adenosine and/or guanosine 3′,5′-cyclic monophosphate (cAMP and/or cGMP) [11]. PDE5 is a cGMP-specific PDE isozyme that terminates the vasodilatory effect of nitric oxide (NO) in smooth muscles by degrading cGMP. Selective PDE5 inhibitors increase intracellular cGMP levels that lead to cGMP-dependent protein kinase (PKG) activation and prolonged vasodilatation [11,12]. Sildenafil (Viagra®) (**B**) and tadalafil (Cialis®) (**C**) are among the most potent PDE5 inhibitors on the market; both drugs are clinically approved for the treatment of male erectile dysfunction and pulmonary arterial hypertension (Fig. 1) [13]. Both drugs were found to have non-selective effects on other PDE isozymes leading to side effects including visual disturbances, headache, flushing, nasal congestion, nasopharyngitis, and dyspepsia. For example, sildenafil inhibits PDE6 due to the high similarity in amino acid sequence as well as secondary structural element of the catalytic domains of PDE5 and PDE6. In addition, tadalafil was shown to inhibit PDE11 [14].

One of the known tools in lead discovery is to enhance a desired minor effect of an existing drug and eliminate its major pharmacological action, an approach known as selective optimization of side activities (SOSA). This approach has the potential to shorten the time and costs to reach more drug-like lead compounds [15]. In our continued efforts to develop novel PDE5 inhibitors [16–20] and based on the reported off-target activity of celecoxib involving PDE5 inhibition, the present work aims to modulate the COX2/PDE5 inhibitory activities of celecoxib to develop a chemically, and potentially biologically distinct class of selective PDE5 inhibitors with reduced side effects.

2. Results and discussion

2.1. Design concept and synthetic strategy

Our initial efforts were aimed at abolishing the COX2 inhibitory activity of celecoxib while keeping the PDE5 inhibition by fine modulation of certain moieties reported to be necessary for COX2 inhibition. This was envisaged mainly by the bioisosteric replacement of the sulfonamide group at the 1-phenyl of celecoxib with a carboxylic acid functionality [21]. It was previously reported that the COX2 inhibitory activity and selectivity of celecoxib and related analogues of the diaryl heterocycle scaffolds are dependent on the presence of the *p*-sulfonamide or *p*-methylsulfonyl groups. The carboxylic acid group was also reported to cause total loss of COX2 activity when it was used as a replacement for the sulfonamide in the related 1,2-diarylcyclopentenes COX-2 inhibitors [22], and significantly reduced the activity in the diaryl pyrazole scaffold [23]. In addition, further modifications at the 1-phenyl but also the 5-phenyl of celecoxib were systematically carried out in order to increase the activity toward PDE5 inhibition. Importantly, the effect of partial saturation of the pyrazole to the Δ^2-pyrazoline on the PDE5 inhibitory activity and on the general PDE isoform selectivity was also investigated. This modification was expected to have a major impact on the inhibitory profile, as it incorporated a tetrahedral kink in the 3D shape. If successful, it would also disrupt the agglomeration of aromatic ring systems, which can lead to disadvantageous pharmacokinetic properties [24]. Further modifications involved the replacement of the electronegative trifluoromethyl group at position 3 of the pyrazole with *t*-butyl. As an alkyl, the *t*-butyl is anticipated to result in reducing COX-2 inhibitory activity [25]. Fig. 2 summarizes the planned modifications.

2.2. Chemistry

Scheme 1 shows the synthesis of the planned pyrazoline derivatives in two steps. In the first step, a Claisen–Schmidt reaction was carried out between pinacolone and aryl aldehydes, using 10% aq KOH in methanol to yield the enone derivatives. In the second step, enones were heated with the respective aryl hydrazine hydrochloride under inert atmosphere to give the desired pyrazoline derivatives. Oxidation of the pyrazoline ring to pyrazole was accomplished via reflux with DDQ in benzene. Two of the carboxylic acid derivatives were converted to the respective ethyl esters using acetyl chloride in refluxing ethanol (Scheme 1).

2.3. Biological evaluation

All the newly synthesized celecoxib analogues were screened for their *in vitro* ability to inhibit human recombinant PDE5, COX1 and COX2 enzymes at concentrations of 50, 100 and 100 μM, respectively. Compounds showing>50% inhibition at the screening dose were tested in a dose–response curve to determine the IC_{50}. Results of PDE5 inhibition are shown in Table 1. All the tested compounds were inactive against COX1 and COX2 enzymes at a concentration of 100 μM except compounds **31**, **32** and **33,** which showed weak inhibition with IC_{50} values of 80, 85 and 34 μM respectively (see Table S1, Supporting information).

2.3.1. Structure-activity relationships for PDE5 inhibition

2.3.1.1. First probe compound and bioisosteric replacement of sulfonamide. Compound **22** was the first compound synthesized in this series by adopting the main structural modifications mentioned above to the structure of celecoxib, where the pyrazole ring is converted to the Δ^2-pyrazoline; the *p*-sulfonamide is converted to a carboxylic acid function; and the trifluoromethyl is converted to *t*-butyl. Compound **22** showed no COX inhibition and a 4-fold improvement in the PDE5 inhibitory activity compared to celecoxib (IC_{50} 8.4 vs. 37 μM, respectively). Moreover, replacing the carboxylic acid function with the more acidic sulfonic acid moiety in compounds **31** and **32** kept the PDE5 inhibitory activity with only about 2-fold reduction in potency (compound **32** compared to **7**). However, these 2 compounds were able to retain some inhibitory activity against COX2 with IC_{50} values of 80 and

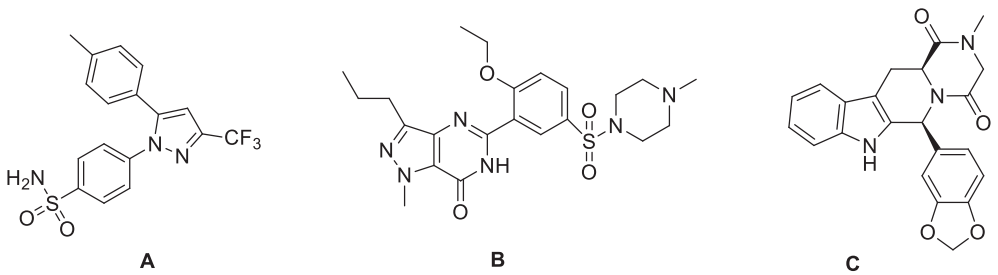


Fig. 1. The chemical structure of celecoxib (A) sildenafil (B) and tadalafil (C).

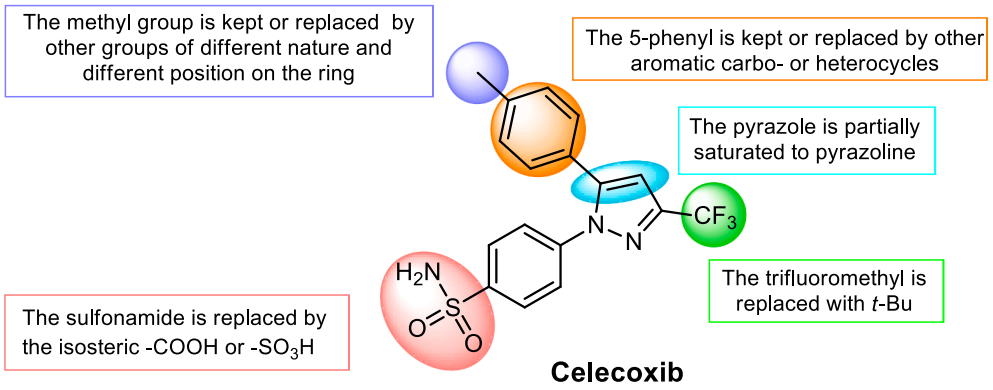
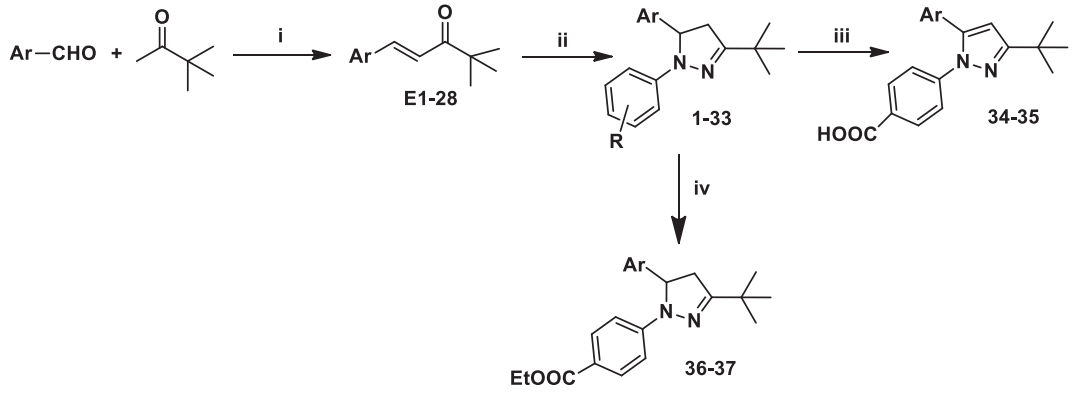


Fig. 2. The modifications adopted to the structure of celecoxib.



Scheme 1. Reagents and conditions: (i) 10%KOH, MeOH, ice cooling then room temperature, overnight (ii) 1.5 equiv Ph-NH-NH_2.HCl derivative, DMF, 85 °C, 5 h. (iii) 1.5 equiv DDQ, benzene, reflux, 5 h. (iv) EtOH, acetyl chloride, reflux, 5 h.

85 μM respectively (Table S1, Supporting information). The suggested modifications including the use of the carboxylic acid functionality fulfilled the required activity shift toward PDE5.

2.3.1.2. Modifications at position 5 of the pyrazoline. The *p*-methyl moiety at the 5-phenyl of compound **22** (that was originally present in celecoxib) was deleted or replaced by other substituents of different electronic, lipophilic or steric nature. In addition, the substitution pattern on ring was varied. In addition, bulkier aromatic systems such as β-naphthyl and biphenyl as well as heteroaromatic systems were tested instead of the 5-phenyl.

Deleting the *p*-methyl in compound **22** gave a slightly more active compound as PDE5 inhibitor (compound **1**, IC_{50} = 6.2 μM). Then, the methyl group in **22** was replaced with several mono halogen substitutions including fluoro, chloro and bromo substituents with different substitution pattern on the ring to give compounds (**2**–**10**). All of the halogen-substituted 5-phenyl analogues showed equivalent or up to 4-fold higher potency to inhibit PDE5 compared to the methyl derivative **22,** with the best activity displayed by compounds **5** and **6** having *ortho* and *meta* chloro substituents (IC_{50} values are 1.9 and 2 μM respectively), while the *p*-fluoro derivative was the least active in this set of monohalogentated compounds (compound 4, IC_{50} = 7.7 μM). Based on this pronounced increase in potency with the mono-chloro derivatives, the dichloro derivatives (**11**–**13**) were synthesized. However, having two chloro substituents on the phenyl did not yield an additive effect in improving the potency to inhibit PDE5. Compounds **11** (with 2,4-dichloro substitution, IC_{50} = 2.7 μM) and **12** (with 3,4-dichloro substitution, IC_{50} = 5.5 μM) were markedly more active than compound **13** (with 2,6- dichloro substitution, IC_{50} = 28.8 μM) which suggested some steric clash that may be caused by this di-*ortho* substitution, preventing the phenyl from getting deeply in a certain pocket. Using the strong electron-withdrawing but less lipophilic nitro group gave compounds of comparable PDE5 inhibitory activity to their halogen congeners (compounds **20** and **21** with IC_{50} values of 3.2 and 5.7 μM respectively). The use of the more hydrophilic cyano moiety at the *para* position led to lower activity than the respective halogens (compound **23**, IC_{50} = 13.8 μM).

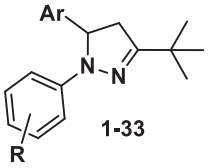
In addition, several electron-donating substituents were evaluated on the 5-phenyl, starting with the methoxy group; indeed, the introduction of the *p*-methoxy substituent gave a 4-fold increase in activity compared to the methyl analogue **22** (compound **15**, IC_{50} = 2 μM). However, moving the methoxy group to the *ortho* position greatly reduced the activity (compound **14,** IC_{50} > 50 μM). This may be attributed to the electronic effect of the oxygen atom in terms of the electronic complementarity with the binding site rather than the steric effect, as it is shown above that the *ortho*-bromo derivative is still showing significant activity (compound **8**, IC_{50} = 6.9 μM). Interestingly, combining both the *ortho* and *para* methoxy groups in compound **16** led to intermediate PDE5 inhibitory activity (IC_{50} = 23.5 μM). By contrast, compound **17** with the 2,5-dimethoxy substituent showed reduced PDE5 inhibitory activity (IC_{50} > 50 μM).

Extending the *p*-methoxy group in compound **15** to the longer chain ethoxy retained PDE5 inhibitory potency (compound 19, IC_{50} = 2.9 μM). Similar to what was observed with the *ortho*-methoxy, the *ortho*-ethoxy in compound **18** showed a high reduction in PDE5 inhibitory potency (compound **18**, IC_{50} > 50 μM).

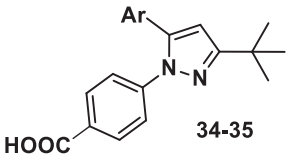
The *p*-hydroxyl moiety (compound **24**, IC_{50} = 2.1 μM) showed comparable PDE5 inhibitory activity to the *p*-methoxy (compound **15**, IC_{50} = 2 μM), with no extra advantage offered by the HBD function.

Furthermore, increasing the bulk of the 5-aryl to biphenyl or

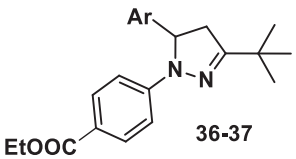
Table 1
PDE5 inhibition by the synthesized compounds.



1-33



34-35



36-37

Cpd #	Ar	R	PDE5 % inh at 50 μM	PDE5 IC_{50}(μM)[a]	Cpd #	Ar	R	PDE5 % inh at 50 μM	PDE5 IC_{50} (μM)[a]
1	phenyl	4-COOH	88	6.2	20	3-nitrophenyl	4-COOH	87.6	3.2
2	2-fluorophenyl	4-COOH	86.3	3.3	21	4-nitrophenyl	4-COOH	91	5.7
3	3-fluorophenyl	4-COOH	94.4	3.7	22	4-methylphenyl	4-COOH	93	8.4
4	4-fluorophenyl	4-COOH	80	7.7	23	4-cyanophenyl	4-COOH	75	13.8
5	2-chlorophenyl	4-COOH	100	1.9	24	4-hydroxyphenyl	4-COOH	77.1	2.1
6	3-chlorophenyl	4-COOH	95.7	2	25	[1,1′-biphenyl]-4-yl	4-COOH	100	35.6
7	4-chlorophenyl	4-COOH	85	3.3	26	naphthalen-2-yl	4-COOH	80	21.8
8	2-bromophenyl	4-COOH	100	6.9	27	furan-2-yl	4-COOH	23.6	ND
9	3-bromophenyl	4-COOH	91.4	3.1	28	thiophen-2-yl	4-COOH	28.1	ND
10	4-bromophenyl	4-COOH	88	7.2	29	4-chlorophenyl	3-COOH	100	9.9
11	2,4-dichlorophenyl	4-COOH	97	2.7	30	4-chlorophenyl	2-COOH	46	ND
12	3,4-dichlorophenyl	4-COOH	100	5.53	31	4-fluorophenyl	4-SO_3H	80	9.3
13	2,6-dichlorophenyl	4-COOH	99.1	28.8	32	4-chlorophenyl	4-SO_3H	85	8.7
14	2-methoxyphenyl	4-COOH	49.8	ND	33	4-chlorophenyl	4-SO_2NH_2	57.5	48.7
15	4-methoxyphenyl	4-COOH	95	2	34	Phenyl	–	72	18.9
16	2,4-dimethoxyphenyl	4-COOH	85.8	23.5	35	4-fluorophenyl	–	75	31.3
17	2,5-dimethoxyphenyl	4-COOH	47	ND	36	4-chlorophenyl	–	10.5	ND
18	2-ethoxyphenyl	4-COOH	42.5	ND	37	3-nitropheyl	–	16	ND
19	4-ethoxyphenyl	4-COOH	95.3	2.9	Celecoxib	–	–	66.5	37

[a] Values are mean of at least two experiments; standard deviation < 12%; ND: not determined, for compounds that showed<50% inhibition at 50 μM, no IC_{50} was determined. Tadalafil was used as a positive control (IC_{50} = 5 nM).

naphthyl (compounds **25** and **26** respectively**),** led to a remarkable drop in activity (IC_{50} values of 35.6 and 21.8 μM respectively) with the bulkier biphenyl showing the lower potency. In comparison to compound **1** with the unsubstituted phenyl (IC_{50} = 6.2 μM), replacing the 5-phenyl with the isosteric furyl (compound **27**) or thienyl (compound **28**) rings was detrimental to the activity. To summarize, the 5-phenyl seemed tolerant to a wide range of substituents with diverse electronic and lipophilic nature, including halogens, nitro, methoxy, ethoxy and

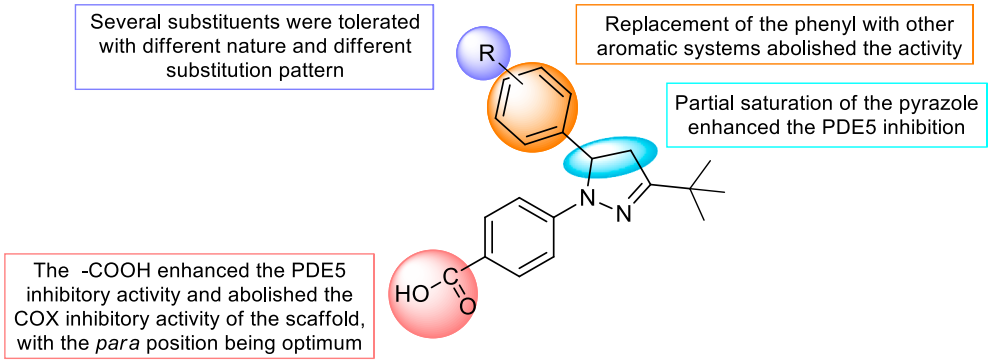


Fig. 3. SAR summary.

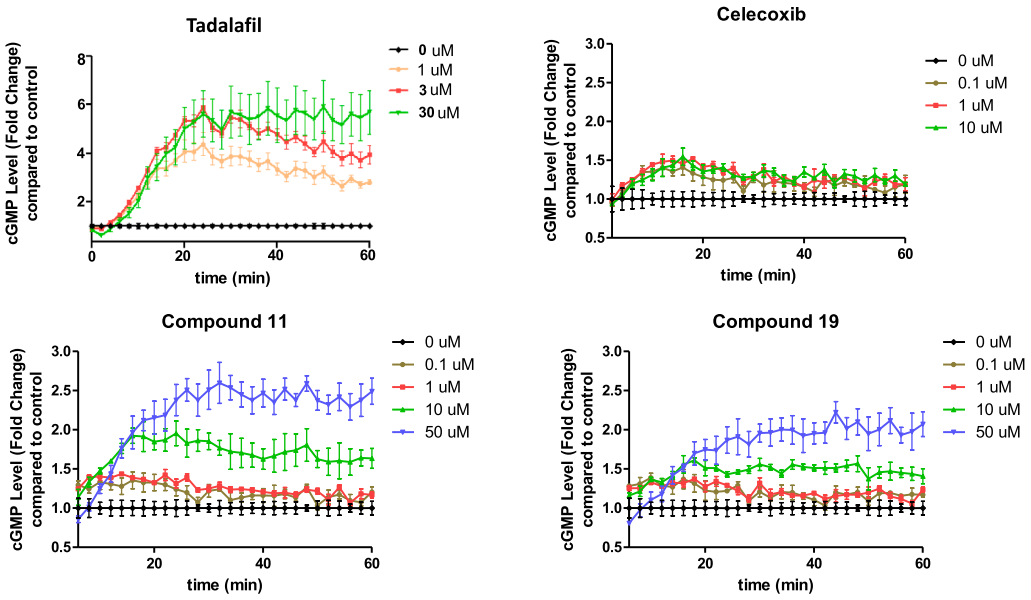


Fig. 4. cGMP elevation in live HEK-293 cells stably expressing a cGMP biosensor. Tadalafil (A), Celecoxib (B), compound 11(C) and compound 19 (D) could show a dose dependent increase in the measured luminescence. All samples were pretreated with sodium nitroprusside to activate the endogenous soluble guanylyl cyclase.

hydroxy substituents, with the substitution pattern more limiting in case of the alkoxy substituents. The phenyl ring at position 5 was irreplaceable by other bulkier aryls or heteroaryl rings.

2.3.1.3. Confirmation of the SAR by further probe compounds. As the main modifications were introduced in combination (replacement of the sulfonamide with –COOH and change the core ring to pyrazoline), we decided to verify the impact of each modification on the PDE5 inhibitory potency.

Replacing the carboxylic acid group in compound **7** with a sulfonamide group reduced the PDE5 inhibitory activity by about 15-fold and partly regained the COX2 inhibition (compound **33**, PDE5 IC_{50} = 48.7 μM, COX2 IC_{50} = 34 μM). Compound **33** confirmed the deleterious effect of the –COOH group on COX2 inhibition and its essential role in PDE5 inhibition. Besides, the *p*-COOH in compound **3** was esterified, or shifted to *o*- or *m*- positions to further test the importance of this group**.** Notably, shifting the –COOH group to the *meta* position led to a 3-fold reduction in the PDE5 inhibitory activity (compound **29**), while shifting it to the *ortho* position diminished the activity (compound **30**); this indicated that the –COOH mediates a critical interaction in the *p*-position that was dramatically influenced by this shift. Esterification of the *p*-COOH moiety with ethyl alcohol in compounds **36** and **37** diminished the PDE5 inhibitory activity, which may be due to the increased size of the substituent, or the loss of the potential polar interactions mediated by the free carboxylate.

Additionally, the importance of the non-coplanar pyrazoline system for the PDE5 inhibitory activity was confirmed by the oxidation of compounds **1** and **4** to the corresponding pyrazole analogues using DDQ to yield compounds **34** and **35**, respectively. Restoring the core ring planarity in the pyrazole system led to a significant reduction in the PDE5 inhibitory activity by 3–4 times (compounds **34** and **35** compared to compounds **1** and **4**). The loss of the core ring planarity in the pyrazoline derivatives with the resulting change in the orientation of the 5-aryl substituent appeared to be one of the main reasons for enhancing the PDE5 inhibition.

Altogether, our probe compound syntheses corroborated that the bioisosteric replacement of the sulfonamide and the partial saturation at the central ring are both essential to the optimization towards selective PDE5 inhibitors. Fig. 3 summarizes the most important SAR conclusions.

2.3.2. Effect on intracellular cGMP levels

Both compounds **11** and **19** were selected for further testing against PDE5 in cells, since both compounds belong to the cluster of the most potent PDE5 inbhiotrs developed in this series (Table S2, Supporting information) and they have the most lipophilic 5-aryl system with electron withdrawing halogen (2,4-dichloro, compound **11**) or electron donating groups (ethoxy, compound **19**). Compounds **11** and **19** showed the highest clogD values compared to the most related potent analogues, thus raising the probability for cell membrane penetration (Table S2, Supporting information); this was estimated to overcome the effect of the highly polar –COOH which may slightly impair cellular permeability. Compounds **11** and **19** along with celecoxib and tadalafil were tested in luciferase cGMP biosensor assay using HEK293 cells stably transfected with a genetically encoded GAF-Luc construct containing a cGMP-specific binding domain from the human PDE5A GAF-A domain fused with modified firefly luciferase. This cGMP biosensor construct measures the relative intracellular cGMP concentration in cells by luminescence. Real-time monitoring of luminescence in the treated cells showed that compounds **11** and **19** and tadalafil as a positive control caused a concentration-dependent increase in intracellular cGMP levels (Fig. 4). In correspondence with their inhibitory effects on recombinant PDE5 activity, celecoxib showed the least effect on cGMP levels, while tadalafil was the most potent and effective. Despite its ability to increase

Table 2
Selectivity profiles of compound **11** vs all PDE isoforms. Compound 11 was screened against all PDE isoforms at 50 μM.[a]

PDE isoform	% inhibition at 50 μM	PDE isoform	% inhibition at 50 μM
PDE1A (cAMP)	2.6	PDE5A (cGMP)	97
PDE1A (cGMP)	22	PDE6C (cGMP)	16.2
PDE2A (cAMP)	5.6	PDE7A (cAMP)	9.1
PDE2A (cGMP)	19.9	PDE8A (cAMP)	4
PDE3A (cAMP)	12.4	PDE9A (cGMP)	10.7
PDE3B (cAMP)	6.7	PDE10A (cAMP)	3.6
PDE3A (cGMP)	36.2	PDE10A (cGMP)	4.5
PDE3B (cGMP)	15.7	PDE11A (cAMP)	0.9
PDE4B2 (cAMP)	1.6	PDE11A (cGMP)	5.9

[a]Values are mean of at least two experiments; standard deviation < 15%.

intracellular cGMP levels, celecoxib did not show a clear concentration-dependent effect, which can be attributed to its lower potency against PDE5. Both compound **11** and **19** showed a substantially higher efficacy in increasing intracellular cGMP levels in HEK239 cells when compared to celecoxib.

2.3.3. PDE isoform selectivity

Since compound **11** showed higher cellular potency than compound **19** (Fig. 4), it **was** selected to be tested for its PDE isozyme selectivity using all 11 recombinant human PDE isozymes. The compound exhibited remarkable selectivity for PDE5 with an IC_{50} of 2.7 μM without significantly affecting the activity of all other PDE isozymes at concentrations up to 50 μM, including PDE6 and PDE11. This suggested that trisubstituted pyrazolines may provide an attractive scaffold for PDE5 inhibition with a promising PDE isozyme selectivity and the potential for fewer side effects. The results are summarized in Table 2.

3. Conclusion

Through systematic structural modifications, the off-target effect of celecoxib to inhibit PDE5 was enhanced while eliminating COX-2 inhibitory activity. The developed trisubstituted pyrazolines represent previously unrecognized scaffold for the development of novel PDE5 inhibitors with the potential for improved isozyme selectivity and reduced side effects. The carboxylic acidic group at the *para* position of the 1- phenyl together with non-coplanar pyrazoline were shown to be essential features to boost the PDE5. Extension of the scaffold might be required to bring the PDE5 inhibitory activity of this novel class to greater potency while retaining PDE5 isozyme selectivity.

4. Experimental section

4.1. Chemistry

Solvents and reagents were obtained from commercial suppliers and used as received without further purification. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker DRX 500 spectrometer, or Varian Mercury 400 spectrometer. The chemical shifts are referenced to the residual protonated solvent signals and occasionally TMS was used as a reference. At least 95% purity in all the tested compounds (Table 1) was obtained using HPLC coupled with mass spectrometry. Mass spectra (HPLC-ESI-MS) were obtained using a TSQ quantum (Thermo Electron Corporation) instrument prepared with a triple quadrupole mass detector (Thermo Finnigan) and an ESI source. All samples were inserted using an autosampler (Surveyor, Thermo Finnigan) by an injection volume of 10 μL. The MS detection was determined using a source CID of 10 V and carried out at a spray voltage of 4.2 kV, a nitrogen sheath gas pressure of $4.0 \cdot 10^5$ Pa, a capillary temperature of 400 °C, a capillary voltage of 35 V and an auxiliary gas pressure of $1.0 \cdot 10^5$ Pa. The stationary phase used was an RP C18 NUCLEODUR 100–3 (125 × 3 mm) column (Macherey&Nagel). The solvent system consisted of water containing 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). HPLC-Method: flow rate of 400 μL/min. The percentage of B started at an initial of 5%, was increased up to 100% during 16 min, kept at 100% for 2 min, and flushed back to 5% in 2 min. Melting points were determined using a Buchi B-540 melting point apparatus and are uncorrected.

4.1.1. General procedure for enone synthesis

The appropriate ketone (10 mmol) was reacted with corresponding aryl aldehyde (10 mmol) using the same procedure we previously reported in ref [26].

4.1.1.1. (E)-4,4-Dimethyl-1-phenylpent-1-en-3-one (E1). Synthesized according to the general procedure for enone synthesis using pinacolone and benzaldehyde; oil; yield: 1.34 g (71%) [27].

4.1.1.2. (E)-1-(2-Fluorophenyl)-4,4-dimethylpent-1-en-3-one (E2). Synthesized according to the general procedure for enone synthesis using pinacolone and *o*-flourobenzaldehyde; oil; yield: 1.48 g (72%) [28].

4.1.1.3. (E)-1-(3-Fluorophenyl)-4,4-dimethylpent-1-en-3-one (E3). Synthesized according to the general procedure for enone synthesis using pinacolone and *m*-fluorobenzaldehyde; yellow solid; yield: 1.4 g (68%); mp 43–44 °C [28].

4.1.1.4. (E)-1-(4-Fluorophenyl)-4,4-dimethylpent-1-en-3-one (E4). Synthesized according to the general procedure for enone synthesis using pinacolone and *p*-fluorobenzaldehyde ; oil; yield: 1.4 g (68%) [29].

4.1.1.5. (E)-1-(2-Chlorophenyl)-4,4-dimethylpent-1-en-3-one (E5). Synthesized according to the general procedure for enone synthesis using pinacolone and *o*-chlorobenzaldehyde; transparent oil; yield: 1.67 g (75%) [28].

4.1.1.6. (E)-1-(3-Chlorophenyl)-4,4-dimethylpent-1-en-3-one (E6). Synthesized according to the general procedure for enone synthesis using pinacolone and *m*-chlorobenzaldehyde; yellow oil; yield: 1.6 g (72%) [28].

4.1.1.7. (E)-1-(4-Chlorophenyl)-4,4-dimethylpent-1-en-3-one (E7). Synthesized according to the general procedure for enone synthesis using pinacolone and *p*-chlorobenzaldehyde; white solid; yield: 1.78 g (80%); mp 85–85.5 °C [30].

4.1.1.8. (E)-1-(2-Bromophenyl)-4,4-dimethylpent-1-en-3-one (E8). Synthesized according to the general procedure for enone synthesis using pinacolone and *o*-bromobenzaldehyde; Yellowish white solid; yield: 2.18 g (82%); mp 49–50 °C [28].

4.1.1.9. (E)-1-(3-Bromophenyl)-4,4-dimethylpent-1-en-3-one (E9). Synthesized according to the general procedure for enone synthesis using pinacolone and *m*-bromobenzaldehyde; Yellow oil; yield: 2.21 g (83%) [28].

4.1.1.10. (E)-1-(4-Bromophenyl)-4,4-dimethylpent-1-en-3-one (E10). Synthesized according to the general procedure for enone synthesis using pinacolone and *p*-bromobenzaldehyde; Yellowish white solid; yield: 2.08 g (78%); mp 101 °C [31].

4.1.1.11. (E)-1-(2,4-Dichlorophenyl)-4,4-dimethylpent-1-en-3-one (E11). Synthesized according to the general procedure for enone synthesis using pinacolone and 2,4-dichlorobenzaldehyde; yellow oil; yield: 2.05 g (80%) [32].

4.1.1.12. (E)-1-(3,4-Dichlorophenyl)-4,4-dimethylpent-1-en-3-one (E12). Synthesized according to the general procedure for enone synthesis using pinacolone and 3,4-dichlorobenzaldehyde; white solid; yield: 2.1 g (82%); mp 89 °C [33].

4.1.1.13. (E)-1-(2,6-Dichlorophenyl)-4,4-dimethylpent-1-en-3-one (E13). Synthesized according to the general procedure for enone synthesis using pinacolone and 2,6-dichlorobenzaldehyde; yellow oil; yield: 2.18 g (85%) [34].

4.1.1.14. (E)-1-(2-Methoxyphenyl)-4,4-dimethylpent-1-en-3-one (E14). Synthesized according to the general procedure for enone synthesis using pinacolone and *o*-anisaldehyde; oil; yield: 1.73 g (79%) [28].

4.1.1.15. (E)-1-(4-Methoxyphenyl)-4,4-dimethylpent-1-en-3-one (E15). Synthesized according to the general procedure for enone synthesis

using pinacolone and *p*-anisaldehyde; oil; yield: 1.64 g (75%) [29].

4.1.1.16. (E)-1-(2,4-Dimethoxyphenyl)-4,4-dimethylpent-1-en-3-one (E16). Synthesized according to the general procedure for enone synthesis using pinacolone and 2,4-dimethoxybenzaldehyde; yellow oil; yield: 1.79 g (72%) [35].

4.1.1.17. (E)-1-(2,5-Dimethoxyphenyl)-4,4-dimethylpent-1-en-3-one (E17). Synthesized according to the general procedure for enone synthesis using pinacolone and 2,5-dimethoxybenzaldehyde; yellow oil; yield: 1.71 g (69%) [35].

4.1.1.18. (E)-1-(2-Ethoxyphenyl)-4,4-dimethylpent-1-en-3-one (E18). Synthesized according to the general procedure for enone synthesis using pinacolone and *o*-ethoxybenzaldehyde; yellow oil; yield: 1.86 g (80%) [29].

4.1.1.19. (E)-1-(4-Ethoxyphenyl)-4,4-dimethylpent-1-en-3-one (E19). Synthesized according to the general procedure for enone synthesis using pinacolone and *p*-ethoxybenzaldehyde; yellow oil; yield: 1.86 g (80%) [36].

4.1.1.20. (E)-4,4-Dimethyl-1-(3-nitrophenyl)pent-1-en-3-one (E20). Synthesized according to the general procedure for enone synthesis using pinacolone and 3-nitrobenzaldehyde; yellow solid; yield: 1.4 g (60%); mp 92–93 °C [37].

4.1.1.21. (E)-4,4-Dimethyl-1-(4-nitrophenyl)pent-1-en-3-one (E21). Synthesized according to the generalprocedure for enone synthesis using pinacolone and *p*-nitrobenzaldehyde; yellow solid; yield: 1.91 g (82%); mp 125–126 °C [38].

4.1.1.22. (E)-4,4-Dimethyl-1-p-tolylpent-1-en-3-one (E22). Synthesized according to the general procedure for enone synthesis using pinacolone and *p*-tolualdehyde; white solid; yield: 1.6 g (79%); mp 79–80 °C [39].

4.1.1.23. (E)-4-(4,4-Dimethyl-3-oxopent-1-enyl)benzonitrile (E23). Synthesized according to the general procedures of enone synthesis using 3,3-dimethylbutan-2-one and 4-formylbenzonitrile; yellow oil; yield: 1.54 g (72%) [40].

4.1.1.24. (E)-1-(4-Hydroxyphenyl)-4,4-dimethylpent-1-en-3-one (E24). Synthesized according to the general procedure for enone synthesis using pinacolone and 4-hydroxybenzaldehyde; yellow oil; yield: 1.84 g (90%) [41].

4.1.1.25. (E)-1-(Biphenyl-4-yl)-4,4-dimethylpent-1-en-3-one (E25). Synthesized according to the general procedure for enone synthesis using pinacolone and 4-biphenylcarbaldehyde; yellow oil; yield: 2.17 g (82%) [42].

4.1.1.26. (E)-4,4-Dimethyl-1-(naphthalene-2-yl)pent-1-en-3-one (E26). Synthesized according to the general procedure for enone synthesis using pinacolone and 2-naphthaldehyde; yellow oil; yield: 1.81 g (76%) [43].

4.1.1.27. (E)-1-(Furan-2-yl)-4,4-dimethylpent-1-en-3-one (E27). Synthesized according to the general procedure for enone synthesis using pinacolone and furan-2-carbaldehyde; yellow oil; yield: 1.32 g (86%) [44].

4.1.1.28. (E)-4,4-Dimethyl-1-(thiophen-2-yl)pent-1-en-3-one (E28). Synthesized according to the general procedure for enone synthesis using pinacolone and thiophene-2-carbaldehyde; yellow oil; yield: 1.53 g (79%) [45].

4.1.2. General procedure for pyrazoline synthesis

A mixture of the enone derivative (2 mmol) and the corresponding aryl hydrazine hydrochloride (3 mmol) were reacted together using the same procedure we previously reported in ref [26].

4.1.2.1. 4-(3-tert-Butyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (1). The title compound was prepared by reaction of 4,4-dimethyl-1-phenylpent-1-en-3-one **(E1)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 38%; mp 233–234 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (s, 1H), 7.55 (d, J = 8.9 Hz, 2H), 7.21 (t, J = 7.5 Hz, 2H), 7.18–7.02 (m, 3H), 6.74 (d, J = 8.9 Hz, 2H), 5.24 (dd, J = 11.7, 5.1 Hz, 1H), 3.48 (dd, J = 17.7, 11.8 Hz, 1H), 2.63 (dd, J = 17.7, 5.1 Hz, 1H), 1.07 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.23, 161.45, 147.80, 142.21, 130.72, 128.97, 127.37, 125.53, 118.95, 111.29, 62.15, 42.54, 33.52, 27.82; MS (ESI): m/z = 323.2 (M^++H).

4.1.2.2. 4-(3-(tert-Butyl)-5-(2-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (2). The title compound was prepared by reaction of 1-(2-fluorophenyl)-4,4-dimethylpent-1-en-3-one **(E2)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); beige powder; yield: 70%; mp 224–226 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 7.68 (d, J = 8.6 Hz, 2H), 7.35–7.18 (m, 2H), 7.08 (dt, J = 26.9, 7.5 Hz, 2H), 6.82 (d, J = 8.5 Hz, 2H), 5.52 (dd, J = 11.8, 4.8 Hz, 1H), 3.62 (dd, J = 17.7, 12.0 Hz, 1H), 2.80 (dd, J = 17.7, 4.8 Hz, 1H), 1.17 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.67, 162.25, 159.86 (d, J = 245.1 Hz), 148.05, 131.32, 130.04 (d, J = 8.2 Hz), 128.76 (d, J = 13.7 Hz), 127.86 (d, J = 4.0 Hz), 125.34 (d, J = 3.3 Hz), 119.67, 116.41 (d, J = 21.0 Hz), 111.63, 56.93, 41.74, 34.03, 28.32; MS (ESI): m/z = 341(M^++H).

4.1.2.3. 4-(3-(tert-Butyl)-5-(3-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (3). The title compound was prepared by reaction of 1-(3-fluorophenyl)-4,4-dimethylpent-1-en-3-one **(E3)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); beige powder; yield: 53%; mp 218–220 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.67 (d, J = 8.7 Hz, 2H), 7.42–7.29 (m, 1H), 7.03 (dt, J = 13.7, 8.0 Hz, 3H), 6.84 (d, J = 8.6 Hz, 2H), 5.39 (dd, J = 11.6, 4.8 Hz, 1H), 3.58 (dd, J = 17.8, 11.8 Hz, 1H), 2.76 (dd, J = 17.8, 4.9 Hz, 1H), 1.17 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.68, 162.82 (d, J = 244.5 Hz), 162.06, 148.14, 145.56 (d, J = 6.6 Hz), 131.62 (d, J = 8.1 Hz), 131.27, 122.02 (d, J = 2.5 Hz), 119.76, 114.74 (d, J = 20.8 Hz), 112.93 (d, J = 21.9 Hz), 111.84, 62.12, 42.85, 34.02, 28.29; MS (ESI): m/z = 341 (M^++H).

4.1.2.4. 4-(3-tert-Butyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzoic acid (4). The title compound was prepared by reaction of 1-(4-fluorophenyl)-4,4-dimethylpent-1-en-3-one **(E4)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 33%; mp 222–224 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.68 (d, J = 9.0 Hz, 2H), 7.24 (tt, J = 5.1, 2.5 Hz, 2H), 7.20–7.11 (m, 2H), 6.86 (d, J = 8.9 Hz, 2H), 5.40 (dd, J = 11.7, 5.0 Hz, 1H), 3.58 (dd, J = 17.7, 11.7 Hz, 1H), 2.75 (dd, J = 17.7, 5.0 Hz, 1H), 1.19 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.21, 161.48, 161.29 (d, J = 243.2 Hz), 147.68, 138.30 (d, J = 2.9 Hz), 130.74, 127.61 (d, J = 8.2 Hz), 119.10, 115.75 (d, J = 21.4 Hz), 111.34, 61.41, 42.45, 33.51, 27.80; MS (ESI): m/z = 341.2 (M^++H).

4.1.2.5. 4-(3-(tert-Butyl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (5). The title compound was prepared by reaction of 1-(2-chlorophenyl)-4,4-dimethylpent-1-en-3-one **(E5)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); orange crystals; yield: 47%; mp 229–231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (d, J = 9.0 Hz, 2H), 7.51 (dd, J = 7.8, 1.4 Hz, 1H), 7.36–7.18 (m, 2H), 6.99–6.86 (m, 1H), 6.75 (d, J = 8.9 Hz, 2H), 5.55 (dd, J = 11.9, 5.2 Hz, 1H), 3.69 (dd, J = 17.8, 11.9 Hz, 1H), 2.71 (dd, J = 17.8, 5.2 Hz, 1H), 1.16 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.66, 162.20, 147.90, 138.87, 131.48, 131.40, 130.48, 129.75, 128.37, 127.20, 119.83, 111.58, 60.20, 41.66, 34.05, 28.32; MS (ESI): m/z = 357 (M^++H).

4.1.2.6. 4-(3-(tert-Butyl)-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (6). The title compound was prepared by reaction of 1-(3-chlorophenyl)-4,4-dimethylpent-1-en-3-one **(E6)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); orange powder; yield: 40%; mp 210–212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 7.68 (d, J = 9.0 Hz, 2H), 7.39–7.28 (m, 2H), 7.26 (t, J = 1.7 Hz, 1H), 7.11 (dt, J = 7.5, 1.3 Hz, 1H), 6.84 (d, J = 8.9 Hz, 2H), 5.39 (dd, J = 11.7, 5.1 Hz, 1H), 3.57 (dd, J = 17.8, 11.8 Hz, 1H), 2.76 (dd, J = 17.8, 5.1 Hz, 1H), 1.16 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.68, 162.09, 148.10, 145.10, 133.93, 131.46, 131.30, 127.91, 126.01, 124.64, 119.79, 111.85, 62.03, 42.85, 34.01, 28.28; MS (ESI): m/z = 357 (M^++H).

4.1.2.7. 4-(3-tert-Butyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzoic acid (7). The title compound was prepared by reaction of 1-(4-chlorophenyl)-4,4-dimethylpent-1-en-3-one **(E7)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white crystals; yield: 41%; mp 208–210 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.24 (s, 1H); 7.74 (d, J = 8.9 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 6.90 (d, J = 8.9 Hz, 2H), 5.45 (dd, J = 11.7, 5.0 Hz, 1H), 3.64 (dd, J = 17.8, 11.8 Hz, 1H), 2.80 (dd, J = 17.7, 5.0 Hz, 1H), 1.23 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.21, 161.51, 147.62, 141.08, 131.89, 130.77, 128.96, 127.53, 119.19, 111.34, 61.43, 42.33, 33.52, 27.79; MS (ESI): m/z = 357.1 (M^++H).

4.1.2.8. 4-(5-(2-Bromophenyl)-3-(tert-butyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (8). The title compound was prepared by reaction of 1-(2-bromophenyl)-4,4-dimethylpent-1-en-3-one **(E8)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); yellow powder; yield: 74%; mp 200–201 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, 1H), 7.73–7.65 (m, 2H), 7.43 (d, J = 4.02 Hz, 1H), 7.29 (d, J = 7.53 Hz, 1H), 7.17–7.15 (m, 2H), 6.89–6.82 (m, 2H), 5.39 (dd, J = 11.37, 5.12 Hz, 1H), 3.59 (dd, J = 17.18, 11.79 Hz, 1H), 2.78 (dd, J = 17.58, 4.59 Hz, 1H), 1.18 (s, 9H); MS (ESI): m/z = 401 (M^++H).

4.1.2.9. 4-(5-(3-Bromophenyl)-3-(tert-butyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (9). The title compound was prepared by reaction of 1-(3-bromophenyl)-4,4-dimethylpent-1-en-3-one **(E9)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); yellow powder; yield: 43%; mp 215–217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 7.71 (d, J = 1.26 Hz, 2H), 7.31 (d, J = 1.35 Hz, 1H), 7.25 (d, J = 1.89 Hz, 1H), 7.22 (d, J = 1.81 Hz, 1H), 7.20–7.19 (m, 1H), 6.94 (d, J = 7.35 Hz, 1H), 6.77–6.72 (m, 1H), 5.50 (dd, J = 11.79, 5.07 Hz, 1H), 3.71(dd, J = 17.77, 11.85 Hz, 1H), 2.70 (dd, J = 17.81, 5.13 Hz, 1H), 1.18 (s, 9H); MS (ESI): m/z = 401 (M^++H).

4.1.2.10. 4-(5-(4-Bromophenyl)-3-tert-butyl-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (10). The title compound was prepared by reaction of 1-(4-bromophenyl)-4,4-dimethylpent-1-en-3-one **(E10)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); yellow crystals; yield: 32%; mp 222–223 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.19 (s, 1H), 7.68 (d, J = 9.0 Hz, 2H), 7.61–7.39 (m, 2H), 7.25–7.08 (m, 2H), 6.84 (d, J = 8.9 Hz, 2H), 5.38 (dd, J = 11.7, 5.0 Hz, 1H), 3.59 (dd, J = 17.8, 11.8 Hz, 1H), 2.75 (dd, J = 17.7, 5.0 Hz, 1H), 1.18 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.20, 161.52, 147.61, 141.50, 131.88, 130.78, 127.88, 120.41, 119.18, 111.34, 61.48, 42.28, 33.52, 27.80; MS (ESI): m/z = 401 (M^++H).

4.1.2.11. 4-(3-(tert-Butyl)-5-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzoic acid (11). The title compound was prepared by reaction of 1-(2,4-dichlorophenyl)-4,4-dimethylpent-1-en-3-one **(E11)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); off- white powder; yield: 40%; mp 213–214 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77–7.67 (m, 3H), 7.36 (dd, J = 8.4, 2.1 Hz, 1H), 6.95 (t, J = 10.3 Hz, 1H), 6.76 (d, J = 8.9 Hz, 2H), 5.55 (dd, J = 11.9, 5.1 Hz, 1H), 3.70 (dd, J = 17.8, 11.9 Hz, 1H), 2.75 (dd, J = 18.0, 5.0 Hz, 1H), 1.17 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.64, 162.33, 147.75, 138.00, 133.32, 132.56, 131.45, 129.99, 128.63, 128.56, 120.04, 111.64, 59.88, 41.45, 34.06, 28.31; MS (ESI): m/z = 391 (M^++H).

4.1.2.12. 4-(3-(tert-Butyl)-5-(3,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (12). The title compound was prepared by reaction of 1-(3,4-dichlorophenyl)-4,4-dimethylpent-1-en-3-one **(E12)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); greenish yellow powder; yield: 90%; mp 216–218 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 (d, J = 9.0 Hz, 2H), 7.63–7.54 (m, 1H), 7.49 (d, J = 2.0 Hz, 1H), 7.12 (dt, J = 8.2, 2.8 Hz, 1H), 6.86 (t, J = 5.7 Hz, 2H), 5.43 (dd, J = 11.7, 5.0 Hz, 1H), 3.58 (dd, J = 17.8, 11.8 Hz, 1H), 2.80 (dd, J = 17.8, 5.1 Hz, 1H), 1.18 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.66, 162.18, 148.00, 143.62, 131.89, 131.77, 131.34, 130.43, 128.37, 126.30, 119.95, 111.91, 61.50, 42.68, 34.02, 28.26; MS (ESI): m/z = 391 (M^++H).

4.1.2.13. 4-(3-(tert-Butyl)-5-(2,6-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (13). The title compound was prepared by reaction of 1-(2,6-dichlorophenyl)-4,4-dimethylpent-1-en-3-one **(E13)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); beige powder; yield: 60%; mp 225–227 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), δ 7.64 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 7.8 Hz, 1H), 7.37 (dt, J = 15.9, 7.9 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 5.89 (dd, J = 13.2, 8.2 Hz, 1H), 3.63 (dd, J = 18.0, 13.3 Hz, 1H), 2.94 (dd, J = 18.1, 8.2 Hz, 1H), 1.22 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.59, 161.68, 148.09, 135.38, 134.75, 134.08, 131.27, 130.75, 129.39, 119.63, 111.22, 59.03, 40.64, 34.18, 28.58; MS (ESI): m/z = 391 (M^+ + H).

4.1.2.14. 4-(3-(tert-Butyl)-5-(2-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (14). The title compound was prepared by reaction of 1-(2-methoxyphenyl)-4,4-dimethylpent-1-en-3-one **(E14)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); yellowish white powder; yield: 70%; mp 217–219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66 (d, J = 8.2 Hz, 2H), 7.22 (s, 1H), 7.05 (d, J = 8.1 Hz, 1H), 6.81 (d, J = 15.7 Hz, 2H), 6.75 (d, J = 8.1 Hz, 2H), 5.50–5.39 (m, 1H), 3.86 (s, 3H), 3.56 (dd, J = 17.4, 12.0 Hz, 1H), 2.71–2.56 (m, 1H), 1.15 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ

167.73, 162.43, 156.40, 148.18, 131.29, 129.27, 129.14, 126.14, 121.08, 119.22, 111.90, 111.45, 57.44, 56.06, 41.76, 34.02, 28.37; MS (ESI): $m/z = 353$ (M^++H).

4.1.2.15. 4-(3-tert-Butyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzoic acid (15). The title compound was prepared by reaction of 1-(4-methoxyphenyl)-4,4-dimethylpent-1-en-3-one **(E15)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 30%; mp 208–209 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.67 (d, J = 9.0 Hz, 2H), 7.15–7.04 (m, 2H), 6.95–6.80 (m,4H), 5.30 (dd, J = 11.7, 5.0 Hz, 1H), 3.70 (s, 3H), 3.55 (dd, J = 17.7, 11.7 Hz, 1H), 2.72 (dd, J = 17.7, 5.0 Hz), 1.19 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.27, 161.46, 158.45, 147.83, 134.12, 130.70, 126.79, 118.87, 114.33, 111.34, 61.66, 55.00, 42.56, 33.55, 27.86; MS (ESI): $m/z = 353.2$ (M^++H).

4.1.2.16. 4-(3-tert-Butyl-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (16). The title compound was prepared by reaction of 1-(2,4-dimethoxyphenyl)-4,4-dimethylpent-1-en-3-one **(E16)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); yellowish green powder; yield: 58%; mp 213–215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 7.66 (d, J = 8.2 Hz, 2H), 6.74 (dd, J = 13.0, 9.0 Hz, 3H), 6.59 (s, 1H), 6.40 (d, J = 8.4 Hz, 1H), 5.37 (dd, J = 11.5, 4.5 Hz, 1H), 3.84 (s, 3H), 3.70 (s, 3H), 3.51 (dd, J = 17.6, 11.8 Hz, 1H), 2.62 (dd, J = 17.7, 4.5 Hz, 1H), 1.15 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.76, 162.37, 160.34, 157.42, 148.17, 131.25, 126.96, 121.49, 119.10, 111.44, 105.41, 99.30, 57.17, 56.12, 55.58, 41.80, 34.01, 28.37; MS (ESI): $m/z = 383.6$ (M^++H).

4.1.2.17. 4-(3-tert-Butyl-5-(2,5-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (17).. The title compound was prepared by reaction of 1-(2,5-dimethoxyphenyl)-4,4-dimethylpent-1-en-3-one **(E17)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); faint yellow powder; yield: 55%; mp 262–264 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (d, J = 8.9 Hz, 2H), 7.00 (d, J = 8.9 Hz, 1H), 6.84–6.74 (m, 3H), 6.38 (d, J = 2.9 Hz, 1H), 5.44 (dd, J = 11.8, 4.9 Hz, 1H), 3.83 (s, 3H), 3.57 (s, 3H), 3.53 (dd, J = 17.7, 11.9 Hz, 1H), 2.68 (dd, J = 17.7, 4.9 Hz, 1H), 1.18 (s, 9H); MS (ESI): $m/z = 383.6$ (M^++H).

4.1.2.18. 4-(3-(tert-Butyl)-5-(2-ethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (18). The title compound was prepared by reaction of 1-(2-ethoxyphenyl)-4,4-dimethylpent-1-en-3-one **(E18)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); greenish yellow powder; yield: 63%; mp 211–212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (d, J = 9.0 Hz, 2H), 7.21 (ddd, J = 8.4, 6.9, 2.2 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1H), 6.90–6.74 (m, 4H), 5.48 (dd, J = 11.8, 5.3 Hz, 1H), 4.23–4.06 (m, 2H), 3.59 (dd, J = 17.7, 11.9 Hz, 1H), 2.68 (dd, J = 17.7, 5.4 Hz, 1H), 1.39 (t, J = 7.0 Hz, 3H), 1.17 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.74, 162.35, 155.63, 148.22, 131.26, 129.59, 129.10, 126.27, 121.06, 119.22, 112.85, 111.44, 64.01, 57.47, 41.77, 34.02, 28.37, 15.20; MS (ESI): $m/z = 367$ (M^++H).

4.1.2.19. 4-(3-(tert-Butyl)-5-(4-ethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (19). The title compound was prepared by reaction of 1-(4-ethoxyphenyl)-4,4-dimethylpent-1-en-3-one **(E19)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); beige powder; yield: 50%; mp 159–160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67–7.64 (m, 2H), 11.82 (s, 1H), 7.11–7.08 (m, 2H), 6.88–6.83 (m, 4H), 5.30 (dd, J = 11.57, 4.97 Hz, 1H), 3.97 (q, J = 6.96 Hz, 2H), 3.56 (dd, J = 17.71, 11.68 Hz, 1H), 2.72 (dd, J = 17.75, 5.01 Hz, 1H), 1.29 (t, J = 6.96 Hz, 3H), 1.19 (s, 9H); MS (ESI): $m/z = 367$ (M^++H).

4.1.2.20. 4-(3-tert-Butyl-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzoic acid (20). The title compound was prepared by reaction of 4,4-dimethyl-1-(3-nitrophenyl)pent-1-en-3-one **(E20)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); yellow powder; yield: 50%; mp 235–237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.10 (d, J = 10.9 Hz, 2H), 7.67 (t, J = 7.7 Hz, 2H), 7.62 (q, J = 8.0 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 5.58 (dd, J = 11.6, 4.7 Hz, 1H), 3.63 (dd, J = 17.8, 11.8 Hz, 2H), 2.82 (dd, J = 17.9, 4.8 Hz, 1H), 1.17 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.64, 162.20, 148.60, 148.01, 144.75, 132.74, 131.35, 131.17, 122.95, 121.10, 120.01, 111.95, 61.85, 42.76, 34.04, 28.27; MS (ESI): $m/z = 367.6$ (M^++H).

4.1.2.21. 4-(3-tert-Butyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzoic acid (21). The title compound was prepared by reaction of 4,4-dimethyl-1-(4-nitrophenyl)pent-1-en-3-one **(E21)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); yellow powder; yield: 35%; mp 245–248 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.21 (s, 1H), 8.27–8.12 (m, 2H), 7.68 (d, J = 8.9 Hz, 2H), 7.47 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.9 Hz, 2H), 5.56 (dd, J = 11.9, 5.1 Hz, 1H), 3.65 (dd, J = 17.8, 11.9 Hz, 1H), 2.79 (dd, J = 17.8, 5.2 Hz, 1H), 1.18 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.16, 161.59, 149.63, 147.51, 146.84, 130.85, 127.05, 124.27, 119.50, 111.41, 61.53, 42.18, 33.56, 27.78; MS (ESI): $m/z = 368.2$ (M^++H).

4.1.2.22. 4-(3-tert-Butyl-5-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (22). The title compound was prepared by reaction of 4,4-dimethyl-1-*p*-tolylpent-1-en-3-one **(E22)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); yellow powder; yield: 35%; mp 194–196 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.67 (d, J = 8.5 Hz), 7.14 (d, J = 7.9 Hz), 7.09 (d, J = 7.9 Hz), 6.86 (d, J = 8.6 Hz), 5.32 (dd, J = 11.7, 5.0 Hz), 3.58 (dd, J = 17.7, 11.8 Hz), 2.73 (dd, J = 18.0, 4.8 Hz), 2.25 (s), 1.19 (s); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.24, 161.43, 147.79, 139.21, 136.52, 130.69, 129.51, 125.45, 118.85, 111.28, 61.91, 42.51, 33.51, 27.84, 20.59; MS (ESI): $m/z = 337.2$ (M^++H).

4.1.2.23. 4-(3-tert-Butyl-5-(4-cyanophenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzoic acid (23). The title compound was prepared by reaction of 4-(4,4-dimethyl-3-oxopent-1-enyl)benzonitrile **(E23)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2:CH_3OH 100:2); white solid; yield: 24%; mp 115–117 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.23 (s, 1H), 7.84–7.79 (m, 2H), 7.71–7.67 (m, 2H), 7.41–7.36 (m, 2H), 6.86–6.81 (m, 2H), 5.50 (dd, J = 11.8, 5.0 Hz, 1H), 3.62 (dd, J = 17.8, 11.9 Hz, 1H), 2.77 (dd, J = 17.8, 5.1 Hz, 1H), 1.18 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.20, 161.61, 147.62, 147.55, 133.04, 130.88, 126.77, 119.46, 118.58, 111.40, 110.32, 61.74, 42.22, 33.58, 27.81; MS (ESI): $m/z = 347.9$ $(M + H)^+$.

4.1.2.24. 4-(3-tert-Butyl-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (24). The title compound was prepared by reaction of 1-(4-hydroxyphenyl)-4,4-dimethylpent-1-en-3-one **(E24)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 65%; mp 259–261 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.66 (d, J = 9.1 Hz, 2H), 7.06–6.93 (m, 2H), 6.92–6.76 (m, 2H), 6.75–6.59 (m, 2H), 5.23 (dd, J = 11.6, 5.1 Hz, 1H), 3.53 (dd, J =

17.7, 11.7 Hz, 1H), 2.70 (dd, J = 17.7, 5.1 Hz, 1H), 1.18 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 167.28, 161.45, 156.59, 147.88, 132.43, 130.67, 126.74, 118.72, 115.63, 111.30, 61.80, 42.58, 33.53, 27.84; MS (ESI): m/z = 339 (M^++H).

4.1.2.25. 4-(5-([1,1′-Biphenyl]-4-yl)-3-(tert-butyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (25). The title compound was prepared by reaction of 1-([1,1′-biphenyl]-4-yl)-4,4-dimethylpent-1-en-3-one **(25)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 45%; mp 232–234 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.15 (s, 1H), 7.76–7.54 (m, 6H), 7.42 (t, J = 7.3 Hz, 2H), 7.37–7.21 (m, 3H), 6.88 (d, J = 8.4 Hz, 2H), 5.40 (dd, J = 11.6, 4.9 Hz, 1H), 3.60 (dd, J = 17.7, 11.8 Hz, 1H), 2.79 (dd, J = 17.7, 4.7 Hz, 1H), 1.18 (s, J = 9.5 Hz, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.71, 162.03, 148.26, 141.84, 140.06, 139.72, 131.26, 129.35, 127.89, 127.76, 127.02, 126.67, 119.48, 111.80, 62.30, 42.96, 34.05, 28.34; MS (ESI): m/z = 399 (M^++H).

4.1.2.26. 4-(3-(tert-Butyl)-5-(naphthalen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (26). The title compound was prepared by reaction of 4,4-dimethyl-1-(naphthalen-2-yl)pent-1-en-3-one **(E26)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 41%; mp 221–223 °C; 1H NMR (400 MHz, DMSO-d_6) δ 7.86 (t, J = 9.1 Hz, 3H), 7.75 (s, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.48 (dd, J = 9.3, 4.8 Hz, 2H), 7.28 (d, J = 8.5 Hz, 1H), 6.89 (d, J = 8.4 Hz, 2H), 5.50 (dd, J = 11.7, 5.2 Hz, 1H), 3.65 (dd, J = 17.8, 11.9 Hz, 1H), 2.82 (dd, J = 17.8, 5.1 Hz, 1H), 1.19 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.69, 162.09, 148.40, 140.16, 133.38, 132.80, 131.22, 129.50, 128.19, 128.02, 126.88, 126.46, 124.68, 124.03, 119.56, 111.83, 62.92, 42.99, 34.07, 28.33; MS (ESI): m/z = 373 (M^++H).

4.1.2.27. 4-(3-tert-Butyl-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (27). The title compound was prepared by reaction of 1-(furan-2-yl)-4,4-dimethylpent-1-en-3-one **(E27)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); faint brown powder; yield: 57%; mp 190–191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 7.72 (d, J = 8.0 Hz, 2H), 7.56 (s, 1H), 7.00 (d, J = 8.1 Hz, 2H), 6.37 (s, 2H), 5.49 (dd, J = 11.6, 4.5 Hz, 1H), 3.44 (dd, J = 17.5, 11.7 Hz, 1H), 3.01 (dd, J = 17.5, 4.4 Hz, 1H), 1.21 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.72, 162.23, 153.43, 148.43, 143.22, 131.12, 119.69, 112.01, 110.80, 107.92, 56.21, 39.05, 34.11, 28.30; MS (ESI): m/z = 312.9 (M^++H).

4.1.2.28. 4-(3-tert-Butyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl) benzoic acid (28). The title compound was prepared by reaction of 4,4-dimethyl-1-(thiophen-2-yl)pent-1-en-3-one **(E28)** and 4-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); dark yellow powder; yield: 58%; mp 214–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, J = 72.6 Hz, 1H), 7.71 (dd, J = 9.2, 2.0 Hz, 2H), 7.41–7.32 (m, 1H), 7.10 (d, J = 2.7 Hz, 1H), 6.98 (t, J = 5.6 Hz, 2H), 6.94 (dd, J = 5.0, 3.5 Hz, 1H), 5.75 (dd, J = 11.2, 4.2 Hz, 1H), 3.55 (dd, J = 17.6, 11.2 Hz, 1H), 2.90 (dd, J = 17.6, 4.3 Hz, 1H), 1.21 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.70, 162.21, 148.33, 145.47, 131.10, 127.18, 125.74, 125.60, 119.92, 112.30, 58.65, 42.99, 34.09, 28.26; MS (ESI): m/z = 328.8 (M^++H).

4.1.2.29. 3-(3-tert-Butyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (29). The title compound was prepared by reaction of 1-(4-chlorophenyl)-4,4-dimethylpent-1-en-3-one **(E7)** and 3-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); faint yellow powder; yield: 61%; mp 245–247 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.70–7.67 (m, 2H), 7.41–7.38 (m, 2H), 7.23–7.20 (m, 2H), 6.86–6.83 (m, 2H), 5.40 (dd, J = 11.7, 5.0 Hz, 1H), 3.58 (dd, J = 17.8, 11.8 Hz, 1H), 2.74 (dd, J = 17.8, 5.0 Hz, 1H), 1.18 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.22, 161.52, 147.63, 141.09, 131.91, 130.79, 128.98, 127.54, 119.18, 111.35, 61.45, 42.36, 33.53, 27.80; MS (ESI): m/z = 356.7 (M^++H).

4.1.2.30. 2-(3-tert-Butyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (30). The title compound was prepared by reaction of 1-(4-chlorophenyl)-4,4-dimethylpent-1-en-3-one **(E7)** and 2-hydrazinobenzoic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 53%; mp 163–165 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H), 7.38–7.33 (m, 2H), 7.32–7.27 (m, 2H), 7.24 (dd, J = 7.6, 1.6 Hz, 1H), 7.10 (ddd, J = 8.7, 7.4, 1.6 Hz, 1H), 6.73 (td, J = 7.5, 0.9 Hz, 1H), 6.54 (d, J = 8.2 Hz, 1H), 5.27 (dd, J = 11.3, 8.3 Hz, 1H), 3.45 (dd, J = 17.3, 11.3 Hz, 1H), 2.71–2.59 (m, 1H), 1.12 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.78, 159.25, 142.61, 141.46, 132.24, 130.42, 129.31, 129.19, 128.54, 123.88, 119.14, 114.67, 63.68, 42.65, 33.96, 28.32; MS (ESI): m/z = 356.7 (M^++H).

4.1.2.31. 4-(3-tert-Butyl-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzenesulfonic acid (31). The title compound was prepared by reaction of 1-(4-fluorophenyl)-4,4-dimethylpent-1-en-3-one **(E4)** and 4-hydrazinobenzene sulfonic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:2); yellow crystals; yield: 25%; mp 92–95 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.65–7.73 (m, 4H), 7.6 (d, J = 8.57 Hz ,2H), 6.75 (d, J = 5.59 Hz, 2H), 5.28 (dd, J = 11.65, 5.76 Hz, 1H), 3.53 (dd, J = 17.49, 11.69 Hz, 1H), 2.69 (dd, J = 17.51, 5.81 Hz, 1H), 1.17 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.73, 146.82, 131.70, 128.63, 127.59, 124.06, 115.68, 115.51, 111.19, 67.39, 62.09, 33.43, 27.91; MS (ESI): m/z = 377.1 (M^++H).

4.1.2.32. 4-(3-tert-Butyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonic acid (32). The title compound was prepared by reaction of 1-(4-chlorophenyl)-4,4-dimethylpent-1-en-3-one **(E7)** and 4-hydrazinobenzene sulfonic acid hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:2); white crystals; yield: 27%; mp 92–95 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.87 (d, J = 9.01 Hz, 2H), 7.44–7.41 (m, 2H), 7.37 (d, J = 8.53 Hz, 2H), 7.34 (d, J = 8.84 Hz, 2H), 5.28 (dd, J = 11.69, 5.80 Hz, 1H), 3.54 (dd, J = 17.53, 11.74 Hz, 1H), 2.69 (dd, J = 17.52, 5.83 Hz, 1H), 1.2 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.57, 144.93, 141.40, 137.93, 131.70, 128.83, 127.70, 126.42, 111.17, 62.14, 42.28, 30.29, 27.91; MS (ESI): m/z = 393 (M^++H).

4.1.2.33. 4-(3-tert-Butyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide (33). The title compound was prepared by reaction of 1-(4-chlorophenyl)-4,4-dimethylpent-1-en-3-one **(E7)** and 4-hydrazinobenzene sulfonamide hydrochloride according to the general procedure for pyrazoline synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 55%; mp 180–182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (t, J = 5.7 Hz, 2H), 7.46–7.32 (m, 2H), 7.27–7.14 (m, 2H), 6.97 (s, 2H), 6.94–6.78 (m, 2H), 5.41 (dd, J = 11.7, 5.0 Hz, 1H), 3.59 (dd, J = 17.7, 11.7 Hz, 1H), 2.76 (dd, J = 17.8, 5.0 Hz, 1H), 1.18 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.96, 147.04, 141.35, 132.80, 132.39, 129.44, 128.06, 127.55, 111.86, 61.91, 42.79, 34.01, 28.28; MS (ESI): m/z = 392.5 (M^++H).

4.1.3. General procedure for pyrazoline oxidation into pyrazole

A mixture of the pyrazoline derivative (1 mmol) and dichlorodicyanoquinone (1.5 mmol) in 10 mL of benzene was heated to reflux for 5 h. The mixture was cooled to room temperature and filtered

through a plug of silica gel wetted with diethyl ether. The filtrate was concentrated *in vacuo* and the residue was purified by CC.

4.1.3.1 4-(3-tert-Butyl-5-phenyl-1H-pyrazol-1-yl)benzoic acid (34). The title compound was prepared by the oxidation of **4-(3-*tert*-butyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzoic acid (1)** according to the general procedure for pyrazole synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 60%; mp 192–194 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.92–7.84 (m, 2H) 7.35–7.23 (m, 7H), 6.6 (s, 1H), 1.24 (s, 9H); MS (ESI): m/z = 321 (M^++H).

4.1.3.2 4-(3- tert-Butyl −5-(4-fluorophenyl)-1H-pyrazol-1-yl) benzoic acid (35). The title compound was prepared by the oxidation of **4-(3-*tert*-butyl-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzoic acid (4)** according to the general procedure for pyrazole synthesis. The product was purified by CC (CH_2Cl_2/CH_3OH 50:1); white powder; yield: 66%; mp180-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93–7.90 (m, 2H), 7.33–7.18 (m, 6H), 6.6 (s, 1H), 1.21 (s, 9H); MS (ESI): m/z = 339 (M^++H).

4.1.4. General procedure for synthesis of ethyl ester derivatives

A mixture of absolute ethanol (10 mL) and acetyl chloride (0.25 mL) was stirred on cold for five minutes, then the corresponding benzoic acid derivative (1 mmol) was added and the mixture was refluxed for 5 h and the residue was purified using CC.

4.1.4.1. Ethyl 4-(3-tert-butyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoate (36). The title compound was prepared by the esterification of **4-(3-*tert*-butyl-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzoic acid (7)** according to the general procedure for ester synthesis. The product was purified by CC (CH_2Cl_2/hexane 4:1); white powder; yield: 50%; mp 144–147 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 (d, J = 9.0 Hz, 2H), 7.45–7.32 (m, 2H), 7.25–7.12 (m, 2H), 6.84 (d, J = 8.9 Hz, 2H), 5.40 (dd, J = 11.7, 4.8 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.58 (dd, J = 17.8, 11.8 Hz, 1H), 2.74 (dd, J = 17.8, 4.9 Hz, 1H), 1.23 (t, J = 7.1 Hz, 3H), 1.16 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.06, 162.35, 148.20, 141.43, 132.39, 131.07, 129.44, 128.02, 118.71, 111.89, 61.81, 60.21, 42.80, 34.04, 28.27, 14.75; MS (ESI): m/z = 384.6 (M^++H).

4.1.4.2. Ethyl 4-(3-tert-butyl-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzoate (37). The title compound was prepared by the esterification of **4-(3-*tert*-butyl-5-(3-nitrophenyl)-4,5-dihydro-*1H*-pyrazol-1-yl) benzoic acid (20)** according to the general procedure for ester synthesis. The product was purified by CC (CH_2Cl_2/hexane 4:1); orange powder; yield: 52%; mp 134–136 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17–8.04 (m, 2H), 7.70 (d, J = 9.0 Hz, 2H), 7.64 (t, J = 7.6 Hz, 1H), 7.60 (dt, J = 7.7, 1.5 Hz, 1H), 6.96–6.83 (m, 2H), 5.62 (dd, J = 11.7, 4.9 Hz, 1H), 4.25–4.10 (m, 2H), 3.64 (dd, J = 17.9, 11.8 Hz, 1H), 2.84 (dd, J = 17.9, 4.9 Hz, 1H), 1.24 (t, J = 7.1 Hz, 3H), 1.19 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.01, 162.52, 148.58, 148.12, 144.62, 132.73, 131.16, 122.96, 121.12, 119.07, 112.01, 61.75, 60.25, 42.74, 34.05, 28.26, 14.73 ; MS (ESI): m/z = 395.7 (M^++H).

4.2. Biological testing

4.2.1. COX assay

COX-1 and COX-2 activities were measured using purified ovine COX-1 and COX-2 with colorimetric assay kits obtained from Cayman Chemical Co. (Ann Arbor, Michigan) according to the manufacturers protocol. Briefly, 10 μL ovine COX-1 and COX-2 and Hemin were diluted in 150 μL assay buffer 0.1 M Tris-HCl, pH 8). Inhibitors or vehicle were added to the reaction in 10 μL of reaction buffer, followed by incubation at 25 °C for 5 min. After the addition of 20 μL the colorimetric substrate, 20 μL arachidonic acid was added and the reaction allowed to proceed at 25 °C for an additional 5 min. Activity was determined by absorbance of the substrate at a wavelength of 590 nm.

4.2.2. PDE assay

PDE activity was measured using the IMAP fluorescence polarization assay (Molecular Devices Inc.) according to the manufacturer's specifications. Activity of recombinant PDE enzymes (BPS Biosciences) was measured through selective binding of the hydrolyzed form (product) of fluorophore labeled cyclic nucleotide substrate to immobilized metal coordination complexes. Binding resulted in increased fluorescence polarization (FP). Tetramethylrhodamine (TAMRA)-cGMP (ex530/em590 nm) and FAM-cAMP (ex485/em530 nm) substrates, each at final concentration of 50 nmol/L were incubated with PDE enzymes at concentrations empirically determined to yield the EC_{80} activity (10 μL, 2X concentrate) in assay buffer (10 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 0.1 mg/mL bovine serum albumin). Inhibitors were incubated with PDE enzymes (5 μL, 4X concentrate in assay buffer) for 60 min at 30 °C, followed by incubation with indicated substrate (5 μL, 4X concentrate in assay buffer) for 30 min at 30 °C. Binding reagent (60 μL) was added and incubated for 10 min at 30 °C. FP was measured at the indicated excitation and emission wavelengths using a Synergy4 (Biotek) microplate reader. IC_{50} values were determined using GraphPad Prism non-linear dose–response software package.

4.2.3. Cyclic GMP biosensor assay

A luciferase cell-based assay to measure changes in intracellular cGMP levels was established by stable transfection of HEK293 cells with a genetically encoded GAF-Luc construct (GloSensor cGMP-40F; Promega) containing a cGMP-specific binding site from the human PDE5A GAF-A domain fused with modified firefly luciferase. Following transfection with Lipofectamine LTX (Invitrogen), stable transfectants were selected with G418 antibiotic. Resulting HEK293-GloSensor cGMP-40F cells were plated at 6×10^4 cells per well in 100 μL per well of 96-well assay plates (Corning) and allowed to attach overnight in Dulbecco's Modification of Eagle's Medium (DMEM; Corning) + 10% fetal bovine serum (FBS; Atlanta Biologicals) at 37 °C and 5% CO_2. Cultures were pre-incubated at room temperature in CO_2 independent media (Gibco) supplemented with 10% FBS and 5 mmol/L luciferin substrate (Promega). For measurement of intracellular cGMP levels, endogenous soluble guanylyl cyclase was activated by addition of 50 μM sodium nitroprusside (SNP; Sigma-Aldrich) in combination with indicated concentrations of test compounds to initiate the experiment. The firefly luciferase domain was activated by the resulting increase of intracellular cGMP binding to the GAF-A domain, and produced bioluminescence values in live cells were measured each minute over a period of 1 h in a Biotek Synergy4 plate reader.

Declaration of competing interest

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

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Appendix A. Supplementary material

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