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Pyrazole-based lamellarin O analogues: synthesis, biological evaluation and structure-activity relationships†

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A library of pyrazole-based lamellarin O analogues was synthesized from easily accessible 3(5)-aryl-1*H*-pyrazole-5(3)-carboxylates which were subsequently modified by bromination, *N*-alkylation and Pd-catalysed Suzuki cross-coupling reactions. Synthesized ethyl and methyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*-pyrazole-5-carboxylates were evaluated for their physicochemical property profiles and *in vitro* cytotoxicity against three human colorectal cancer cell lines HCT116, HT29, and SW480. The most active compounds inhibited cell proliferation in a low micromolar range. Selected ethyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*-pyrazole-5-carboxylates were further investigated for their mode of action. Results of combined viability staining *via* Calcein AM/Hoechst/PI and fluorescence-activated cell sorting data indicated that cell death was triggered in a non-necrotic manner mediated by mainly G2/M-phase arrest.

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Introduction

Lamellarins are a group of natural marine-derived alkaloids with a characteristic central pyrrole moiety and widely reported biological activity. Since their discovery in 1985, more than 50 compounds of this family have been isolated from various marine organisms, mainly, but not exclusively, sponges and ascidians.¹⁻³ Most lamellarins contain 6H[1]benzo-pyrano [4',3':4,5]pyrrolo[2,1-*a*]isoquinolinone chromophore and fall into two subtypes – 5,6-saturated and 5,6-unsaturated lamellarins, designated as type Ia and type Ib, respectively. Structurally less complex type II lamellarins form a smaller class of compounds bearing 3,4-diarylpyrrole-2-carboxylate fragment (Fig. 1).^{4,5}

The biological spectrum of lamellarins is manifold, with pronounced cytotoxic activity.⁶⁻¹¹ In detail, lamellarin D (Fig. 1), a leading compound in the family of type I, induces its

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Fig. 1 Relevant lamellarin alkaloid representatives of type I and type II bearing a characteristic central pyrrole core and novel pyrazole containing analogues.

anticancer activity through topoisomerase I inhibition and mitochondrial targeting that triggers cell death.¹²⁻¹⁵ Lamellarin O was investigated by Huang *et al.*¹⁶ As reported, cytotoxicity effects of some natural lamellarins were assessed towards colorectal cancer SW620 and its multi-drug resistant daughter cell line SW620/Ad300. Lamellarin O exhibited moderate cytotoxicity against aforementioned cells at IC₅₀ of 20.0 μ M and 22.3 μ M respectively. Whereas lukianol A, a natural cyclized lactone of lamellarin O, is known to exhibit cytotoxicity against human epidermoid carcinoma cell lines.¹⁷ Other lamellarins can reverse multidrug resistance and consequently promote therapeutic activity of conventional cytotoxic drugs towards chemoresistant tumours.^{5,18}

The unbridled key goal is, besides appropriate strategies to synthetically approach these natural compounds,^{17,19-21} to create new analogues as innovative drug-like compounds with potent antitumor activities.²²⁻²⁴ Zheng et al. reported synthesis and investigation of novel glycosylated lamellarin D compounds wherein glycosyl moieties improve important physicochemical properties of active compounds, especially the solubility in water.25 Another study revealed A-ring modified lamellarin N analogues as potent noncovalent inhibitors of EGFR T790M/ L858R mutant, which is responsible for non-small cell lung cancer resistance.26 More recent investigation on A-ring modified azalamellarins revealed that synthetic analogues selectively inhibit the proliferation of EGFR T790M/L858R mutant cells over EGFR WT cells.27 Moreover, Klumthong et al. presented a diversity-oriented synthesis of azalamellarins, where lactoneto-lactam modification resulted in increased cytotoxicity against HeLa cervical cancer cells.28

Following these significant discoveries, interest in lamellarin based research has been growing and remains highly relevant. The structure of lamellarin O is easily accessible for replacement of the central pyrrole ring to design new derivatives with structural similarities like shape and electronic configuration by other five membered ring systems. Pyrazole is a versatile moiety taking place in various biologically active compounds as well as in-use pharmaceuticals.²⁹⁻³¹ Replacement of the central pyrrole to pyrazole is expected to change *e.g.*, the energy of the highest occupied molecular orbital (HOMO) which is associated with increased metabolic stability.³²

In continuation of our previous works devoted to synthesis and investigation of pyrazole derivatives,^{33–41} in this study we ought to synthesize and investigate various functionalized pyrazole derivatives of lamellarin O. The goal was based on the scaffold hopping of the pyrrole ring in natural lamellarin O to its pyrazole counterpart. Synthetic strategy involves 3,5substituted pyrazole formation and pyrazole functionalization at 4-position by Pd-catalysed Suzuki cross-coupling reaction. Obtained compounds were evaluated for their physicochemical properties and further investigated as potent agents against human colon cancer cell lines HCT116, HT29 and SW480. Moreover, after structure–activity relationship determination, the most cytotoxic compounds were used to investigate their mode of action in the before mentioned cell lines.

Results and discussion

Chemistry

As outlined in Scheme 1, Claisen condensation of commercially available acetophenones **1a–c** with diethyl oxalate and subsequent cyclocondensation with hydrazine hydrate afforded 3(5)-aryl-1*H*-pyrazole-5(3)-carboxylates **2a–c** in good yields as previously described by Wu *et al.*⁴²

For introduction of phenyl substituents at C-4 of pyrazole ring, a halogen atom at the indicated position was introduced beforehand. 3(5)-Aryl-1*H*-pyrazole-5(3)-carboxylates **2a–c** underwent bromination reaction using NBS in DCM and products **3a–c** were obtained in 88–93% yields.⁴³ Thereafter, two alternative pathways could be employed – either by first forming



Scheme 1 Synthesis of intermediates 4a-k. Reagents and conditions: (i) diethyl oxalate, NaOEt, EtOH, rt, 16 h;⁴² (ii) NH₂NH₂·H₂O, AcOH, EtOH, rt, 16 h;⁴² (iii) NBS, DCM, 35 °C, 16 h;⁴³ (iv) appropriate 2-bromoacetophenone, Na₂CO₃, DMF, 60–70 °C, 5–8 h.

Paper

C-C bond via cross-coupling reaction and subsequently conducting N-alkylation, or vice versa. Multiple experiments were carried out towards Suzuki cross-coupling with pyrazole 3a bearing free -NH group (see ESI, Table S1[†]). Unfortunately, most C-C bond formation reactions resulted in complex mixtures and product yield did not exceed 22%. To tackle this problem, functionalization of free -NH group had to be performed in the first place.44

It is known that NH-pyrazoles usually exhibit annular N,Nprototropy.45 Typically, N-alkylation of asymmetrically ringsubstituted 1H-pyrazoles results in the formation of a mixture of regioisomeric N-substituted products,46-49 therefore regioselective N-alkylation requires optimisation of reaction conditions. In this work, the goal was to carry out alkylation of 3(5)aryl-4-bromo-1*H*-pyrazole-5(3)-carboxylates 3a-c in a regioselective manner to obtain desired isomers 4a-k as major products. Experiments were carried out with 3(5)-aryl-1H-pyrazole-5(3)-carboxylate 3a and the influence of the solvent and/or



Fig. 2 ¹H (red), ¹³C (italic), ¹⁵N (blue) NMR chemical shifts and relevant ¹³C-HMBC, ¹H, ¹⁵N-HMBC, ¹H, ¹H-NOESY correlations of ¹H. regioisomers 4a and 5a.

base on the regiochemical outcome of the reaction was evaluated. Comparison of Na₂CO₃, K₂CO₃ and NaH bases using DMF or ACN as a solvent revealed that combination of Na₂CO₃ and less polar DMF gave the best regioselectivity ratio of isomers 4a and 5a. Therefore, reaction conditions with Na₂CO₃-DMF system were applied for the synthesis of intermediates 4a-k.

N-Alkylated products 4a and 5a were fully characterized based on ¹H, ¹H-COSY, ¹H, ¹H-NOESY, ¹H, ¹³C-HSQC, ¹H, ¹³Cand ¹H, ¹⁵N-HMBC experimental data (Fig. 2). To assign the regiochemistry of isomers, ¹H, ¹³C-HMBC experiment was fundamental. Obtained data of regioisomer 4a revealed strong heteronuclear three-bond correlation between NCH₂ protons at δ 6.23 ppm and annular C-5 of pyrazole at δ 132.1 ppm. Another correlation for distinguishing regioisomers is a long-range coupling between the same NCH₂ protons at δ 6.23 ppm and carboxylate ester carbon at δ 158.1 ppm. Minor isomer 5a can be easily identified in a similar manner. A strong three-bond coupling was observed between NCH₂ protons at δ 5.94 ppm and pyrazole C-5 at δ 144.5 ppm. Distinct NOE was observed between NCH₂ protons at δ 5.94 and 5-phenyl ring 2(6)-H protons at δ 7.35–7.41 ppm, which confirms their proximity in space. The absence of heteronuclear correlation between NCH₂ protons and carbon atom from carboxylate ester indicates the structure of 5-phenyl-1H-pyrazole-3-carboxylate 5a.

Synthesized 3-aryl-4-bromo-1H-pyrazole-5-carboxylate intermediates 4a-k were used for the final derivatization step i.e., construction of C-C bond via Pd-catalysed Suzuki crosscoupling reaction. As the efficiency and yield of transition metal catalysed reactions are influenced by various factors such as catalysts, solvents, bases, ligands, or other additives, 50-53 optimization was carried out beforehand using carboxylate 4a as a model compound (Table 1). For the C–C coupling $Pd(PPh_3)_4$ was employed as a catalyst. Investigation started with the use of K₃PO₄ in DMF and water as a co-solvent for the dissolution of inorganic base. In the initial attempt, reaction mixture was

Table 1 Optimization of Suzuki cross-coupling reaction conditions using 4a as a model compound



stirred at 100 °C for 16 h affording hydrolysed product 1-(2-oxo-2-phenylethyl)-3,4-diphenyl-1*H*-pyrazole-5-carboxylic acid (**6a**') in 53% yield (Table 1, entry 1). As reaction with K_3PO_4 in DMF/ H_2O resulted in successful C–C bond formation, it was repeated using MW-assisted heating (Table 1, entry 2). In the latter experiment hydrolysed product was once again isolated as a sole reaction product, however, the outcome of the reaction has improved and reached 77% yield.

Further investigations involved multiple experiments using K_3PO_4 in less polar dioxane or dioxane/water solvent systems. Using conventional heating (Table 1, entry 3) both desired carboxylate ester **6a** and hydrolysed product **6a**' were isolated in 51% and 38% yields, respectively. To our satisfaction, reaction with K_3PO_4 in dioxane/water system under MW irradiation proceeded without the undesired hydrolysis, giving rise to **6a** in 78% yield (Table 1, entry 4). Additional experiment using saturated Na₂CO₃ solution in toluene/EtOH mixture⁵⁴ was carried out giving very low 16% yield of **6a** (Table 1, entry 5). Few more reactions were investigated using Cs₂CO₃ as a base (Table 1, entry 8), entries 6 and 7) and Pd(OAc)₂ as a catalyst (Table 1, entry 8),

revealing $Pd(PPh_3)_4$ and Cs_2CO_3 -dioxane-H₂O system as the most suitable approach for C–C bond formation. In contrast to a cross-coupling under conventional heating, MW-assisted heating dramatically shortened reaction times, formed relatively pure products, and increased overall yields. Therefore, MW irradiation took a significant role in successful formation of the target compounds.

Optimized Suzuki cross-coupling reaction conditions were applied to evaluate the scope of the reaction and to couple phenyl-, 4-methoxyphenyl- and 4-fluorophenylboronic acids with *N*-alkylated pyrazole-5-carboxylates **4a–k** (Scheme 2). Microwave-assisted Suzuki cross-coupling was successfully exploited to synthesize a library of ethyl 3,4-diaryl-1-(2-aryl-2oxoethyl)-1*H*-pyrazole-5-carboxylates **6a–o** in 73–97% yields. Additionally, three 3,4-diaryl-1*H*-pyrazole-5-carboxylates **6a–c** underwent transesterification in the presence of K₂CO₃ in refluxing methanol to derive methyl carboxylates **7a–c** in 92– 96% yields.



Scheme 2 Synthesis of target pyrazole core-bearing lamellarin O analogues. Reagents and conditions: (i) appropriate phenylboronic acid, Pd(PPh₃)₄, Cs₂CO₃, dioxane, H₂O, MW, 100 °C, 1 h; (ii) K₂CO₃, MeOH, reflux, 3 h.

Table 2Comparison of HPLC-log P and $c \log P$ data of ethyl andmethyl3,4-diaryl-1-(2-aryl-2-oxoethyl)-1H-pyrazole-5-carboxylates6a-o and 7a-c

| Compound | HPLC-log P^a | $c \log P^b$ |
|--------------|-------------------|--------------|
| Lamellarin O | _ | 4.00 |
| Lukianol A | _ | 4.57 |
| Lamellarin D | | 3.79 |
| Lamellarin I | | 4.17 |
| 6a | 4.381 ± 0.002 | 5.28 |
| 6b | 4.468 ± 0.006 | 5.15 |
| 6c | 4.453 ± 0.005 | 5.44 |
| 6d | 4.711 ± 0.006 | 5.84 |
| 6e | 3.803 ± 0.012 | 4.89 |
| 6f | 4.462 ± 0.005 | 5.02 |
| 6g | 4.531 ± 0.004 | 4.90 |
| 6h | 4.531 ± 0.006 | 5.18 |
| 6i | 4.784 ± 0.005 | 5.58 |
| 6j | 4.490 ± 0.006 | 5.44 |
| 6k | 4.546 ± 0.005 | 5.59 |
| 6l | 4.499 ± 0.006 | 5.31 |
| 6m | 4.545 ± 0.006 | 5.59 |
| 6n | 4.564 ± 0.004 | 5.47 |
| 60 | 4.598 ± 0.004 | 5.75 |
| 7a | 4.279 ± 0.005 | 4.94 |
| 7b | 4.347 ± 0.007 | 4.81 |
| 7 c | 4.327 ± 0.007 | 5.10 |

 a Data is provided as a mean value \pm standard deviation (SD) of at least three independent experiments. b Calculated using ChemDraw 13.0.

Physicochemical properties

Estimation of the drug-likeness is facilitated by the evaluation of physicochemical properties and their causal relationships to predict pharmacokinetics. Despite the development of the rule of five reported by Lipinski *et al.*⁵⁵ to identify key properties for

potential bioavailability in drug design, natural products and drugs based on naturally occurring compounds were not explicitly included in this systematization.⁵⁶

The lipophilicity of compounds 6a-o and 7a-c was estimated using both, high throughput chromatographic method employing an octadecyl-poly(vinyl alcohol) stationary phase57 and computational methods (Table 2). Besides, topological polar surface area (tPSA), pK_a of strongest acid as well as hydrogen bond donors (HBD) and acceptors (HBA) were calculated (see ESI, Table S2^{\dagger}). The calculated log P ($c \log P$) and measured HPLC-log P were in a narrow range of 4.81-5.84 and 3.803-4.784, respectively, with the hydroxy-substituted derivative 6e being the least lipophilic one. All natural products, lamellarin O, lamellarin D, lamellarin I and lukianol A had a lower lipophilicity in the range of 3.79-4.57 for $c \log P$. A similar trend was observed for the calculated tPSA values, where the pyrazole-based derivatives presented a reduced polarity. The N-substitution pattern on the pyrazole ring, based on the structural similarity to lamellarin O, was the main reason for the increase of lipophilicity. Once the nitrogen in position 1 is substituted, the pyrazole loses the amphoteric properties⁵⁸ in parallel to the lower HOMO energy of the pyrazole versus pyrrole ring.32

Taken together, being as close as possible to the structure of lamellarin O, all pyrazole-based derivatives are outliers of Lipinski's rule of five. However, it is reported that natural products and compounds derived from natural products are among the most favourable exceptions from the Lipinski's rule of five.⁵⁹

Biological evaluation

Synthesized ethyl and methyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*pyrazole-5-carboxylates **6a–o** and **7a–c** were first evaluated for their cytotoxicity against three human colorectal cancer cell

| Table 3 In vitro cytotoxicity of synthesized compounds | | | | | | | | | | |
|--|-------------------|-------------|-------|----------------|-------|---|-------------------|-------------------|--|--|
| Compound | General structure | R^1 R^2 | | | R^4 | $\mathrm{GI}_{50}\pm\mathrm{SD}^{a}\left(\mu\mathrm{M} ight)$ | | | | |
| | | | R^2 | R ³ | | HCT116 | HT29 | SW480 | | |
| 6a | | Н | Н | Н | Et | 5.462 ± 0.393 | >20 | >20 | | |
| 6b | | н | OMe | н | Et | >20 | >20 | >20 | | |
| 6c | | Н | F | Н | Et | 1.964 ± 0.266 | 4.979 ± 2.620 | 5.523 ± 3.719 | | |
| 6d | | Н | Cl | Н | Et | 14.459 ± 3.915 | >20 | >20 | | |
| 6e | | Н | OH | Н | Et | >20 | >20 | >20 | | |
| 6f | R ³ | OMe | Н | OMe | Et | 2.293 ± 0.218 | >20 | >20 | | |
| 6g | | ОМе | ОМе | ОМе | Et | >20 | >20 | >20 | | |
| 6h | R ¹ | OMe | F | OMe | Et | 2.072 ± 1.450 | 4.759 ± 2.331 | 4.759 ± 2.331 | | |
| 6i | COOR4 | ОМе | Cl | ОМе | Et | >20 | >20 | >20 | | |
| 6j | | F | Н | Н | Et | 3.751 ± 1.056 | 13.757 ± 0.861 | 9.243 ± 2.147 | | |
| 6k | R ² | F | Н | F | Et | 3.386 ± 0.620 | 11.125 ± 2.619 | 7.807 ± 2.017 | | |
| 61 | | F | Н | OMe | Et | 5.051 ± 1.164 | >20 | 8.275 ± 2.339 | | |
| 6m | | F | F | н | Et | 1.456 ± 0.247 | 2.688 ± 0.110 | 1.441 ± 0.173 | | |
| 6n | | F | F | OMe | Et | 2.097 ± 0.198 | 6.783 ± 0.463 | 1.641 ± 0.022 | | |
| 60 | | F | F | F | Et | 2.362 ± 0.039 | 6.666 ± 2.121 | 2.088 ± 0.032 | | |
| 7a | | н | н | н | Ме | >20 | >20 | >20 | | |
| 7 b | | н | OMe | н | Ме | >20 | >20 | >20 | | |
| 7 c | | Н | F | Н | Me | 2.699 ± 0.689 | 9.725 ± 3.711 | >20 | | |

 a Data is provided as a mean value \pm standard deviation (SD) of at least three independent experiments.

lines HCT116, HT29 and SW480 (Table 3). Six out of eighteen compounds did not reach GI_{50} in the given 20 μ M concentration range because of their wide therapeutic window and low solubility in cell culture medium. Ethyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*-pyrazole-5-carboxylates **6c**, **h**, **j**, **k**, **m–o**, and methyl 1-[2-(4-fluorophenyl)-2-oxoethyl]-3,4-diphenyl-1*H*-py-razole-5-

carboxylate (7c) have shown activity in the low micromolar range towards all tested cell lines. In most of the cases compounds were more active towards HCT116 cells, except for ethyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*-pyrazole-5-carboxylates **6m–o** which have shown slightly higher cytotoxicity against SW480 cells. As expected, compounds bearing fluorine substituent demonstrated the best results.⁶⁰ Fluorine substitution at R² had the highest impact on advantageous cytotoxicity, whereas fluorine substitution on R¹ and R³ had only minor or no effects. Among them, compound **6m** proved to be the most active and reached lowest GI₅₀ concentrations of 1.456 μ M, 2.688 μ M, and 1.441 μ M towards HCT116, HT29 and SW480 cells, respectively.

Comparing synthesized ethyl carboxylates **6a–c** with their methyl esters **7a–c** it was noticed that ethyl group positively affects activity of the compounds. Interestingly, compound **6a** appeared to be selective towards HCT116, and **6c**, bearing fluorine atom at 4'-position of acetophenone, was active against all tested cell lines. In comparison, compounds **7a**, **b** showed no activity at all, whereas methyl carboxylate **7c** was less active than its counterpart **6c**.

Influence of the substituent at 4'-position (Fig. 3) was evaluated in pyrazoles having identical substituents at 3- and 4position. Interestingly, ethyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*pyrazole-5-carboxylates **6a** and **6f** with no substituents at 4'position were more active compared to those possessing Cl, OH or OMe groups and, as anticipated, fluorine substituent improved cytotoxicity of both **6c** and **6h** towards all cell lines. Additionally, presence of OMe group at both 4- and 4"-positions of compound **6f** improved activity against HCT116 cells two-fold compared to carboxylate **6a**. In case of ethyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*-pyrazole-5-carboxylates **6j**, **1**, **m**, **n** containing fluorine atom, it was noticed that compounds with different substituents at 3,4-positions do not lose their activity. On the contrary, most active compound **6m** possessed 4-fluorophenyl and phenyl substituents in the 3- and 4-positions of the pyrazole ring respectively.

The significant cytotoxicity of pyrazole-based derivatives motivated us to investigate the mode of action of the most potent compounds. First, we evaluated the compounds by a conventional live/dead counterstain including propidium iodide (PI), Hoechst 33258 and Calcein AM.⁶¹ Calcein AM is a well-described cell-permeable dye staining living cells, whereas PI can only bind to DNA when the cellular membrane is disrupted and allows the visualization of necrotic cells. Hoechst 33258 stain intensity increases during apoptosis due to nuclear condensation indicating apoptosis. The absence of any PI staining of the cells indicated the absence of necrotic and late-apoptotic cells for all tested compounds in all three colorectal cancer cells (ESI Fig. S134–S136†).

Cell cycle distribution was determined using flow cytometry in order to verify if chosen ethyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1H-pyrazole-5-carboxylates 6c and 6m could induce cell cycle arrest. HCT116 cells were treated for 72 h with the respective compounds in a dose-dependent manner. As shown in Fig. 4, treatment of HCT116 cells with target compounds resulted in an induction of G1 and predominantly G2/M cell cycle arrest for both compounds already for the lowest concentration of 5 μ M. Compared to negative control cells, the G1 phase increased significantly from 49.5 \pm 1.8% to 63.2 \pm 1.4% and 61.0 \pm 1.8% for 6c and 6m respectively. In parallel, the G2/M phase increased from 14.2 \pm 1.1% to 25.0 \pm 0.5% and 26.9 \pm 0.8%, respectively. No significant increase of cell debris was observed over the whole concentration range. Cell cycle arrest in the G2 phase was also described for lamellarin D in P388 leukaemia cells,62 whereas to the best of our knowledge no mode of action studies were published for lamellarin O. However, it seems likely that our newly synthesized isosteric analogues of lamellarin O and lamellarin D exhibit comparable cytotoxicity and cellular effects.

Additionally, plasma protein binding (PPB) experiments by means of a high-throughput HPLC method applying albuminbound stationary phase were performed with selected ethyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*-pyrazole-5-carboxylates **6a**, **c**,



Fig. 3 Structure and activity comparison of compounds 6k, 6m, and 7c.



Fig. 4 Cell cycle analysis in HCT116 cells after treatment with 6c and 6m: (a) dose-dependent increase of G1 and G2/M phase for 6c; (b) dose-dependent increase of G1 and G2/M phase for 6m; (c) representative histograms for the negative control, and at 20 μ M of 6c and 6m. Data is provided as mean value of at least three independent experiments.

f, **h**, **m–o**. Compounds **6a**, **c**, **m–o** showed very strong (above 95%) binding to human serum albumin (see ESI, Table S3†). The relationship between %PPB and calculated log *P* values correlated – increased compound lipophilicity results in stronger binding to albumin. However, studied compounds bind differently than would have been expected only from their lipophilicity, as the HPLC–log *P* values did not show linear correlation with %PPB data. High PPB decreases the free plasma fraction of the drug being part of the free drug hypothesis stating that only the unbound fraction of a drug can unfold their biological efficacy.⁶³ This hypothesis is opposing the evidence of albumin being a versatile drug carrier *via* the enhanced permeability and retention (EPR) effect for oncological issues and that 45% of newly approved drugs between 2003 and 2013 possessed PPB of >95%.⁶⁴

Materials and methods

Chemistry

General. The reagents and solvents were purchased from commercial suppliers and used without further purification unless indicated otherwise. Microwave-assisted reactions were conducted using a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC, USA). The purification of the reaction mixtures was performed using flash chromatography on a glass column with silica gel (high-purity grade (9385), 60 Å, 230–400 mesh, Merck KGaA, Darmstadt, Germany). For thin layer chromatography, ALUGRAM® pre-coated TLC plates (Silica gel 60 F254, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) were

employed. Melting points were determined using DigiMelt MPA160 apparatus (Nyköping, Sweden) and are uncorrected. The IR spectra were recorded on a Brüker TENSOR 27 (Brüker Optik GmbH, Ettlingen, Germany) spectrometer using KBr pellets. NMR spectra were recorded using Brüker Avance III spectrometer (400 MHz for ¹H NMR, 100 MHz for ¹³C NMR, 40 MHz for ¹⁵N NMR; Brüker BioSpin AG, Fallanden, Switzerland) at 25 °C. Residual solvent signals were used as internal standards, *i.e.*, for DMSO-d₆ $\delta^{1}_{H} = 2.50$ and $\delta^{13}_{C} = 39.52$, for CDCl₃ $\delta^{1}_{H} = 7.26$ and $\delta^{13}_{C} = 77.16$, for acetone-d₆ $\delta^{1}_{H} = 2.05$ and $\delta^{13}_{C} =$ 29.84. A neat external nitromethane standard was used to recalculate ¹⁵N chemical shifts. The full and unambiguous assignments of the ¹H, ¹³C, ¹⁵N-NMR resonances were achieved using standard Brüker software and a combination of advanced NMR spectroscopic techniques. High-resolution mass spectra were recorded on a micrOTOF-Q III Brüker spectrometer (Brüker Daltonik GmbH, Bremen, Germany) in electrospray ionization mode.

Synthesis of ethyl 3(5)-aryl-1*H*-pyrazole-5(3)-carboxylates 2ac (ref. 42). To a 0.5 M solution of sodium ethoxide (1.1 eq.) in ethanol, appropriate acetophenone 1a-c (1 eq.) and diethyl oxalate (1 eq.) were added, and resulting mixture was stirred at room temperature for 16 h under argon atmosphere. Upon completion, reaction mixture was quenched with 1 M HCl solution until neutral pH and extracted with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (Hex/EtOAc 15/1, v/v). Resulting ketoester (1 eq.) was dissolved in a mixture of EtOH/AcOH (7/3, v/v) to obtain 0.2 M solution. Hydrazine hydrate (1.1 eq.; 55% aqueous solution) was added, and the reaction mixture was stirred at room temperature for 16 h. Subsequently, solvents were evaporated, the resulting residue was dissolved in EtOAc and washed with 10% aqueous NaHCO₃ solution and brine, sequentially dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (Hex/EtOAc/MeOH gradient from 8/1/0.1 to 1/1/0.1, v/v/v) to give corresponding pyrazoles **2a-c** in 83–87% yield.

Synthesis of ethyl 3(5)-aryl-4-bromo-1*H*-pyrazole-5(3)carboxylates 3a-c (ref. 43). An appropriate pyrazole 2a-c (1 eq.) was dissolved in DCM (0.2 M), NBS (1.5 eq.) was added, and the reaction mixture was stirred at 35 °C for 16 h. After full conversion of the starting material reaction mixture was washed with water and brine. Organic phase was separated, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Crude was purified by column chromatography (gradient from Hex/EtOAc 8/1 to 4/1, v/v) to give products 3a-c in 88–93% yield.

Synthesis of ethyl 3-aryl-4-bromo-1-(2-arylethyl-2-oxo)-1*H*pyrazole-5-carboxylates 4a–k, 5a. An appropriate pyrazole 3a–c (1 mmol) was dissolved in dry DMF (0.7 M), Na₂CO₃ (2 mmol), corresponding 2-bromoacetophenone (1.05 mmol) was added, and the reaction mixture was stirred at 70 °C for 8 h. After full conversion of the starting material, reaction mixture was diluted with EtOAc and washed with water and brine. Organic phase was separated, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Crude was purified by column chromatography to yield products 4a–k, 5a.

Ethyl 4-bromo-1-(2-oxo-2-phenylethyl)-3-phenyl-1H-pyrazole-5carboxylate (4a). White solid, yield 62% (256 mg). $R_{\rm f} = 0.69$ (nhexane/ethyl acetate 7/3, v/v), mp 93–94 °C. IR (KBr) ν_{max} cm⁻¹: 2982, 2952, 1704 (C=O), 1448, 1269, 1091, 688. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 1.15 (t, J = 7.1 Hz, 3H, CH₃), 4.23 (q, J = 7.1 Hz, 2H, OCH₂), 6.23 (s, 2H, NCH₂), 7.43–7.54 (m, 3H, 3-Ph 3,4,5-H), 7.58-7.64 (m, 2H, C(O)Ph 3,5-H), 7.71-7.77 (m, 1H, C(O)Ph 4-H), 7.79-7.84 (m, 2H, 3-Ph 2, 6-H), 8.06-8.10 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 13.7 (CH₃), 60.3 (NCH₂), 61.5 (OCH₂), 97.0 (C-4), 127.8 (3-Ph C-2,6), 128.1 (C(O)Ph C-2,6), 128.6 (3-Ph C-3,5), 128.7 (3-Ph C-4), 129.0 (C(O)Ph C-3,5), 130.9 (3-Ph C-1), 132.1 (C-5), 134.1 (C(O) Ph C-1), 134.2 (C(O)Ph C-4), 148.4 (C-3), 158.1 (COO), 192.7 (C= O). ¹⁵N NMR (40 MHz, DMSO-d₆) δ_N ppm: -175.9 (N-1), -63.2 (N-2). HRMS (ESI) for $C_{20}H_{17}BrN_2NaO_3$ ([M + Na]⁺): calcd m/z435.0315, found *m*/*z* 435.0316.

Ethyl 4-bromo-1-[2-(4-methoxyphenyl)-2-oxoethyl]-3-phenyl-1Hpyrazole-5-carboxylate (4b). White solid, yield 60% (266 mg). $R_{\rm f} =$ 0.61 (*n*-hexane/ethyl acetate 7/3, v/v), mp 131–132 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3035, 3006, 2977, 2947, 1714 (C=O), 1601, 1449, 1174, 694. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 1.26 (t, J = 7.1 Hz, 3H, CH₃), 3.82 (s, 3H, OCH₃), 4.26 (q, J = 7.1 Hz, 2H, OCH₂), 5.95 (s, 2H, NCH₂), 6.87–6.95 (m, 2H, C(O)Ph 3,5-H), 7.28–7.41 (m, 3H, 3-Ph 3,4,5-H), 7.75–7.82 (m, 2H, 3-Ph 2,6-H), 7.86–7.94 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 14.1 (CH₃), 55.7 (OCH₃), 59.7 (NCH₂), 61.8 (OCH₂), 98.3 (C-4), 114.3 (C(O)Ph C-3,5), 127.6 (C(O)Ph C-1), 128.44 (3-Ph C-3,5), 128.46 (3-Ph C-2,6), 128.7 (3-Ph C-4), 130.4 (C(O)Ph C-2,6), 131.5 (3-Ph C-1), 132.7 (C-5), 150.0 (C-3), 159.4 (COO), 164.3 (C(O)Ph C-4), 190.1 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -179.4 (N-1), -65.7 (N-5). HRMS (ESI) for C₂₁H₁₉BrN₂NaO₄ ([M + Na]⁺): calcd *m/z* 465.0420, found *m/z* 465.0423.

4-bromo-1-[2-(4-fluorophenyl)-2-oxoethyl]-3-phenyl-1H-Ethyl *pyrazole-5-carboxylate (4c).* White solid, yield 70% (302 mg). $R_{\rm f} =$ 0.64 (n-hexane/ethyl acetate 7/1, v/v), mp 96-97 °C. IR (KBr) v_{max} , cm⁻¹: 3071, 2985, 2946, 1704 (C=O), 1595, 1090, 836, 699. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 1.35 (t, J = 7.1 Hz, 3H, CH₃), 4.34 (q, J = 7.1 Hz, 2H, OCH₂), 6.03 (s, 2H, NCH₂), 7.16–7.24 (m, 2H, C(O)Ph 3,5-H), 7.37-7.49 (m, 3H, 3-Ph 3,4,5-H), 7.82-7.89 (m, 2H, 3-Ph 2,6-H), 7.99-8.06 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR $(101 \text{ MHz}, \text{CDCl}_3) \delta_{\text{C}} \text{ ppm: } 14.1 (\text{CH}_3), 59.8 (\text{NCH}_2), 61.9 (\text{OCH}_2),$ 98.4 (C-4), 116.38 (d, ${}^{2}J_{CF} = 22.1$ Hz, C(O)Ph C-3,5), 128.44 (3-Ph C-2,6), 128.47 (3-Ph C-3,5), 128.8 (3-Ph C-4), 130.84 (d, ${}^{3}J_{CF} =$ 9.5 Hz, C(O)Ph C-2,6), 131.05 (d, ${}^{4}J_{CF} = 3.1$ Hz, C(O)Ph C-1), 131.41 (3-Ph C-1), 132.5 (C-5), 150.2 (C-3), 159.4 (COO), 166.40 $(d, J_{CF} = 256.4 \text{ Hz}, C(O)\text{Ph C-4}), 190.3 (C=O).$ ¹⁵N NMR (40 MHz, $CDCl_3$) δ_N ppm: -180.0 (N-1), -65.7 (N-2). HRMS (ESI) for C_{20} - $H_{16}BrFN_2NaO_3$ ([M + Na]⁺): calcd m/z 453.0221, found m/z453.0218.

4-bromo-1-[2-(4-chlorophenyl)-2-oxoethyl]-3-phenyl-1H-Ethyl *pyrazole-5-carboxylate (4d).* White solid, yield 70% (313 mg). $R_{\rm f} =$ 0.77 (n-hexane/ethyl acetate 7/3, v/v), mp 87-88 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3061, 2983, 2941, 1707 (C=O), 1580, 1226, 1092, 697. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 1.35 (t, J = 7.1 Hz, 3H, CH_3 , 4.33 (q, J = 7.1 Hz, 2H, OCH_2), 6.01 (s, 2H, NCH_2), 7.38-7.52 (m, 5H, 3-Ph 3,4,5-H; C(O)Ph 3,5-H), 7.84-7.89 (m, 2H, 3-Ph 2,6-H), 7.90-7.95 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, $CDCl_3$) δ_C ppm: 14.1 (CH₃), 59.8 (NCH₂), 61.9 (OCH₂), 98.4 (C-4), 128.41 (3-Ph C-2,6), 128.46 (3-Ph C-3,5), 128.8 (3-Ph C-4), 129.47 (C(O)Ph C-3,5), 129.48 (C(O)Ph C-2,6), 131.4 (3-Ph C-1), 132.5 (C-5), 132.9 (C(O)Ph C-1), 140.7 (C(O)Ph C-4), 150.1 (C-3), 159.4 (COO), 190.7 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -180.1 (N-1), -65.6 (N-2). HRMS (ESI) for C₂₀H₁₆BrClN₂NaO₃ ([M + Na]⁺): calcd m/z 468.9925, found m/z 468.9923.

Ethyl 4-bromo-1-[2-(4-hydroxyphenyl)-2-oxoethyl]-3-phenyl-1H*pyrazole-5-carboxylate (4e)*. White solid, yield 56% (240 mg). $R_{\rm f} =$ 0.63 (n-hexane/ethyl acetate 1/1, v/v), mp 191-192 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3377 (OH), 2984, 2945, 1707 (C=O), 1580, 1176, 1090, 837. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 1.15 (t, J =7.1 Hz, 3H, CH₃), 4.22 (q, J = 7.1 Hz, 2H, OCH₂), 6.10 (s, 2H, NCH₂), 6.88-6.96 (m, 2H, C(O)Ph 3,5-H), 7.41-7.54 (m, 3H, 3-Ph 3,4,5-H), 7.76-7.83 (m, 2H, 3-Ph 2,6-H), 7.91-7.99 (m, 2H, C(O) Ph 2,6-H), 10.60 (s, 1H, OH). ¹³C NMR (101 MHz, DMSO-d₆) δ_C ppm: 13.7 (CH₃), 60.0 (NCH₂), 61.5 (OCH₂), 96.84 (C-4), 115.6 (C(O)Ph C-3,5), 125.6 (C(O)Ph C-1), 127.8 (3-Ph C-2,6), 128.6 (3-Ph C-3,5), 128.7 (3-Ph C-4), 130.8 (C(O)Ph C-2,6), 131.0 (3-Ph C-1), 132.3 (C-5), 148.3 (C-3), 158.1 (COO), 163.0 (C(O)Ph C-4), 190.5 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm N}$ ppm: -175.1 (N-1), -63.4 (N-2). HRMS (ESI) for $C_{20}H_{17}BrN_2NaO_4$ ([M + Na]⁺): calcd *m*/*z* 451.0264, found *m*/*z* 451.0265.

Ethyl 4-bromo-3-(4-methoxyphenyl)-1-(2-oxo-2-phenylethyl)-1Hpyrazole-5-carboxylate (4f). White solid, yield 63% (279 mg). $R_{\rm f} =$ 0.79 (*n*-hexane/ethyl acetate 7/3, v/v), mp 152–153 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3068, 2955, 2835, 1704 (C=O), 1455, 1251, 1177,

Paper

1093, 1029, 841. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 1.14 (t, *J* = 7.1 Hz, 3H, CH₃), 3.81 (s, 3H, OCH₃), 4.22 (q, *J* = 7.0 Hz, 2H, OCH₂), 6.19 (s, 2H, NCH₂), 7.01–7.11 (m, 2H, 3-Ph 3,5-H), 7.56–7.65 (m, 2H, C(O)Ph 3,5-H), 7.70–7.79 (m, 3H, C(O)Ph 4-H; 3-Ph 2,6-H), 8.03–8.11 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 13.7 (CH₃), 55.2 (OCH₃), 60.3 (NCH₂), 61.5 (OCH₂), 96.7 (C-4), 114.0 (3-Ph C-3,5), 123.3 (3-Ph C-1), 128.1 (C(O)Ph C-2,6), 129.0 (C(O)Ph C-3,5), 129.2 (3-Ph C-2,6), 132.0 (C-5), 134.15 (C(O)Ph C-1), 134.23 (C(O)Ph C-4), 148.3 (C-3), 158.1 (COO), 159.6 (3-Ph C-4), 192.8 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm N}$ ppm: –177.1 (N-1), –64.3 (N-2). HRMS (ESI) for C₂₁H₁₉BrN₂NaO₄ ([M + Na]⁺): calcd *m/z* 465.0420, found *m/z* 465.0418.

Ethyl 4-bromo-3-(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-2oxoethyl]-1H-pyrazole-5-carboxylate (4g). White solid, yield 56% (265 mg). $R_{\rm f} = 0.59$ (*n*-hexane/ethyl acetate 7/3, v/v), mp 109– 110 °C. IR (KBr) ν_{max} , cm⁻¹: 2991, 2945, 2839, 1709 (C=O), 1689, 1234, 1176, 1030, 840. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 1.33 (t, J = 7.1 Hz, 3H, CH₃), 3.85 (s, 3H, Ph 4-OCH₃), 3.89 (s, 3H, C(O)Ph 4-OCH₃), 4.32 (q, J = 7.0 Hz, 2H, OCH₂), 6.00 (s, 2H, NCH₂), 6.92-7.04 (m, 4H, C(O)Ph 3,5-H; 3-Ph 3,5-H), 7.74-7.85 (m, 2H, 3-Ph 2,6-H), 7.93-8.00 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 14.1 (CH₃), 55.4 (3-Ph 4-OCH₃), 55.7 (C(O)Ph 4-OCH₃), 59.6 (NCH₂), 61.8 (OCH₂), 98.0 (C-4), 113.9 (3-Ph C-3,5), 114.3 (C(O)Ph C-3,5), 124.1 (3-Ph C-1), 127.6 (C(O)Ph C-1), 129.8 (3-Ph C-2,6), 130.4 (C(O)Ph C-2,6), 132.5 (C-5), 149.8 (C-3), 159.4 (COO), 160.0 (3-Ph C-4), 164.3 (C(O)Ph C-4), 190.2 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -180.3 (N-1), -67.0 (N-2). HRMS (ESI) for $C_{22}H_{21}BrN_2NaO_5$ ([M + Na]⁺): calcd m/z495.0526, found m/z 495.0523.

Ethyl 4-bromo-1-[2-(4-fluorophenyl)-2-oxoethyl]-3-(4-methoxyphenyl)-1H-pyrazole-5-carboxylate (4h). White solid, yield 65% (230 mg). $R_{\rm f} = 0.73$ (*n*-hexane/ethyl acetate 3/2, v/v), mp 157– 158 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3083 and 3073 (doublet), 2951, 2934, 2837, 1704 (C=O), 1598, 1268, 839, 611 and 591 (doublet). ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 1.37 (t, *J* = 7.1 Hz, 3H, CH₃), 3.87 (s, 3H, OCH₃), 4.35 (q, *J* = 7.0 Hz, 2H, OCH₂), 6.04 (s, 2H, NCH₂), 6.96-7.03 (m, 2H, 3-Ph 3,5-H), 7.18-7.26 (m, 2H, C(O)Ph 3,5-H), 7.78-7.85 (m, 2H, 3-Ph 2,6-H), 8.01-8.08 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 14.1 (CH₃), 55.4 (OCH₃), 59.8 (NCH₂), 61.9 (OCH₂), 98.2 (C-4), 113.9 (3-Ph C-3,5), 116.37 (d, ${}^{2}J_{CF} = 22.1$ Hz, C(O)Ph C-3,5), 124.0 (3-Ph C-1), 129.8 (3-Ph C-2,6), 130.83 (d, ${}^{3}J_{CF} = 9.5$ Hz, C(O)Ph C-2,6), 131.09 (d, ${}^{4}J_{CF} = 3.0 \text{ Hz}, C(O)Ph C-1), 132.4 (C-5), 150.0 (C-3), 159.5 (COO),$ 160.1 (3-Ph C-4), 166.39 (d, $J_{CF} = 256.3$ Hz, C(O)Ph C-4), 190.4 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -180.9 (N-1). -67.1 (N-2). HRMS (ESI) for $C_{21}H_{18}BrFN_2NaO_4$ ([M + Na]⁺): calcd m/z483.0326, found *m*/*z* 483.0332.

Ethyl 4-bromo-1-[2-(4-chlorophenyl)-2-oxoethyl]-3-(4-methoxyphenyl)-1H-pyrazole-5-carboxylate (4i). White solid, yield 63% (301 mg). $R_{\rm f} = 0.70$ (n-hexane/ethyl acetate 7/3, v/v), mp 119– 120 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 2993, 2952, 1705 (C=O), 1252, 1177, 1092, 1025, 842. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 1.15 (t, J = 7.1 Hz, 3H, CH₃), 3.81 (s, 3H, OCH₃), 4.22 (q, J =7.1 Hz, 2H, OCH₂), 6.19 (s, 2H, NCH₂), 7.01–7.10 (m, 2H, 3-Ph 3,5-H), 7.66–7.77 (m, 4H, C(O)Ph 3,5-H; 3-Ph 2,6-H), 8.05–8.11 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 13.7 (CH₃), 55.2 (OCH₃), 60.2 (NCH₂), 61.5 (OCH₂), 96.7 (C-4), 114.0 (3-Ph C-3,5), 123.3 (3-Ph C-1), 129.14 (3-Ph C-2,6), 129.17 (C(O)Ph C-3,5), 130.0 (C(O)Ph C-2,6), 131.9 (C-5), 132.9 (C(O)Ph C-1), 139.2 (C(O)Ph C-4), 148.3 (C-3), 158.1 (COO), 159.6 (3-Ph C-4), 192.0 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm N}$ ppm: -177.2 (N-1), -64.5 (N-2). HRMS (ESI) for C₂₁H₁₈BrClN₂NaO₄ ([M + Na]⁺): calcd *m/z* 499.0031, found *m/z* 499.0029.

Ethyl 4-bromo-3-(4-fluorophenyl)-1-(2-oxo-2-phenylethyl)-1H*pyrazole-5-carboxylate (4j).* White solid, yield 70% (302 mg). $R_{\rm f} =$ 0.70 (n-hexane/ethyl acetate 7/3, v/v), mp 97-98 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3001 and 2982 (doublet), 2938, 2360, 2342, 1708 (C=O), 1445, 1255, 1226, 1165, 844, 756, 688. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 1.34 (t, J = 7.1 Hz, 3H, CH₃), 4.33 (q, J =7.1 Hz, 2H, OCH₂), 6.06 (s, 2H, NCH₂), 7.09-7.18 (m, 2H, 3-Ph 3,5-H), 7.49-7.57 (m, 2H, C(O)Ph 3,5-H), 7.61-7.69 (m, 1H, C(O) Ph 4-H), 7.81-7.89 (m, 2H, 3-Ph 2,6-H), 7.97-8.03 (m, 2H, C(O) Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 14.1 (CH₃), 60.0 (NCH_2) , 61.9 (COO), 98.2 (C-4), 115.49 (d, ${}^2J_{CF} = 21.6$ Hz, 3-Ph C-3,5), 127.60 (d, ${}^{4}J_{CF} = 3.3$ Hz, 3-Ph C-1), 128.1 (C(O)Ph C-2,6), 129.2 (C(O)Ph C-3,5), 130.31 (d, ${}^{3}J_{CF} = 8.4$ Hz, 3-Ph C-2,6), 132.7 (C-5), 134.3 (C(O)Ph C-4), 134.6 (C(O)Ph C-1), 149.2 (C-3), 159.30 (COO), 163.12 (d, $J_{CF} = 248.1$ Hz, 3-Ph C-4), 191.7 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) δ_N ppm: -179.8 (N-1), -66.0 (N-2). HRMS (ESI) for $C_{20}H_{16}BrFN_2NaO_3$ ([M + Na]⁺): calcd m/z453.0221, found *m*/*z* 453.0225.

4-bromo-3-(4-fluorophenyl)-1-[2-(4-fluorophenyl)-2-oxo-Ethyl ethyl]-1H-pyrazole-5-carboxylate (4k). White solid, yield 69% (310 mg). $R_f = 0.77$ (*n*-hexane/ethyl acetate 3/2, v/v), mp 99–100 °C. IR (KBr) ν_{max} , cm⁻¹: 3084, 2989, 2943, 1708 (C=O), 1598, 1443, 1233, 841. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 1.15 (t, J =7.1 Hz, 3H, CH₃), 4.23 (q, J = 7.0 Hz, 2H, OCH₂), 6.21 (s, 2H, NCH₂), 7.31-7.39 (m, 2H, 3-Ph 3,5-H), 7.41-7.49 (m, 2H, C(O)Ph 3,5-H), 7.81-7.88 (m, 2H, 3-Ph 2,6-H), 8.13-8.20 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) δ_C ppm: 13.7 (CH₃), 60.3 (NCH₂), 61.6 (OCH₂), 96.9 (C-4), 115.59 (d, ${}^{2}J_{CF} = 21.7$ Hz, 3-Ph C-3,5), 116.15 (d, ${}^{2}J_{CF} = 22.0$ Hz, C(O)Ph C-3,5), 127.38 (d, ${}^{4}J_{CF} =$ 3.2 Hz, 3-Ph C-1), 129.99 (d, ${}^{3}J_{CF} = 8.5$ Hz, 3-Ph C-2,6), 130.89 (d, ${}^{4}J_{CF} = 2.8$ Hz, C(O)Ph C-1), 131.24 (d, ${}^{3}J_{CF} = 9.7$ Hz, C(O)Ph C-2,6), 132.2 (C-5), 147.6 (C-3), 158.0 (COO), 162.28 (d, $J_{\rm CF}$ = 246.0 Hz, 3-Ph C-4), 165.59 (d, J_{CF} = 253.0 Hz, C(O)Ph C-4), 191.39 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm N}$ ppm: -175.9 (N-1), -63.7 (N-2). HRMS (ESI) for C₂₀H₁₅BrF₂N₂NaO₃ ([M + Na]⁺): calcd m/z 471.0126, found m/z 471.0130.

Ethyl 4-bromo-1-(2-oxo-2-phenylethyl)-5-phenyl-1H-pyrazole-3carboxylate (5a). Pale yellow solid, yield 19% (79 mg). $R_{\rm f} =$ 0.35 (*n*-hexane/ethyl acetate 7/3, v/v), mp 148–149 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 2981, 2952, 1714 (C=O), 1460 and 1451 (doublet), 1226, 1054. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 1.31 (t, J =7.1 Hz, 3H, CH₃), 4.33 (q, J = 7.1 Hz, 2H, OCH₂), 5.94 (s, 2H, NCH₂), 7.35–7.41 (m, 2H, 3-Ph 2,6-H), 7.45–7.56 (m, 5H, 3-Ph 3,4,5-H; C(O)Ph 3,5-H), 7.65–7.71 (m, 1H, C(O)Ph 4-H), 7.89– 7.95 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 14.1 (CH₃), 57.8 (NCH₂), 60.7 (OCH₂), 95.8 (C-4), 127.1 (3-Ph C-1), 128.1 (C(O)Ph C-2,6), 128.90 (3-Ph C-3,5), 128.94 (C(O)Ph C-3-5), 129.6 (3-Ph C-2,6), 129.9 (3-Ph C-4), 133.8 (C(O) Ph C-1), 134.4 (C(O)Ph C-4), 139.5 (C-3), 144.5 (C-5), 160.5 (COO), 192.7 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm N}$ ppm: -175.1 (N-1), -63.4 (N-2). HRMS (ESI) for C₂₀H₁₇BrN₂NaO₃ ([M + Na]⁺): calcd *m*/*z* 435.0315, found *m*/*z* 435.0313.

General procedure for synthesis of 1-(2-oxo-2-phenylethyl)-3,4-diphenyl-1*H*-pyrazole-5-carboxylic acid (6a'). Ethyl 4-bromo-1-(2-oxo-2-phenylethyl)-3-phenyl-1*H*-pyrazole-5-carboxylate 4a (0.5 mmol) was dissolved in degassed mixture of DMF and water (5/1, v/v, 0.03 M), phenylboronic acid (0.6 mmol), K₃PO₄ (1.5 mmol) and Pd(PPh₃)₄ (0.025 mmol) were added, and reaction mixture was stirred in MW reactor at 140 °C for 1 h. Reaction mixture was filtered through the pad of celite. Filtrate was diluted with EtOAc and washed with water and brine. Organic phase was separated, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. Crude was purified by column chromatography (gradient from Hex/EtOAc 8/1 to 1/ 2, v/v) to yield coupled product 6a'.

1-(2-Oxo-2-phenylethyl)-3,4-diphenyl-1H-pyrazole-5-carboxylic acid (6a'). White solid, yield 77% (147 mg). $R_{\rm f} = 0.46$ (ethyl acetate), mp 209–210 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3058, 3029, 2989, 2940, 2556, 1716 (C=O), 1702 (C=O), 1449, 1228, 1089, 700. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 6.21 (s, 2H, CH₂), 7.19–7.42 (m, 10H, 3-Ph 2,3,4,5,6-H; 4-Ph 2,3,4,5,6-H), 7.56-7.65 (m, 2H, C(O)Ph 3,5-H), 7.68-7.78 (m, 1H, C(O)Ph 4-H), 8.03-8.13 (m, 2H, C(O)Ph 2,6-H), 13.16 (s, 1H, COOH). ¹³C NMR (101 MHz, DMSOd₆) δ_C ppm: 59.3 (CH₂), 123.8 (C-4), 127.3 (4-Ph C-4), 127.5 (4-Ph C-2,6), 127.7 (3-Ph C-4), 128.0 (3-Ph C-2,6), 128.1 (3-Ph C-3,5), 128.2 (C(O)Ph C-2,6), 129.0 (C(O)Ph C-3,5), 130.4 (4-Ph C-3,5), 132.2 (3-Ph C-1), 132.4 (C-5), 133.0 (4-Ph C-1), 134.0 (C(O)Ph C-4), 134.5 (C(O)Ph C-1), 147.8 (C-3), 160.8 (COOH), 193.2 (C= O). ¹⁵N NMR (40 MHz, DMSO-d₆) δ_N ppm: -177.9 (N-1), -65.8 (N-2). HRMS (ESI) for $C_{24}H_{19}N_2O_3$ ([M + H]⁺): calcd m/z383.1390, found *m*/*z* 383.1389.

General procedure for synthesis of ethyl 3,4-diaryl-1-(2-aryl-2-oxoethyl)-1*H*-pyrazole-5-carboxylates (6a–o). An appropriate compound 4a–k (0.5 mmol) was dissolved in degassed mixture of dioxane and water (5/1, v/v, 0.03 M), appropriate aryl boronic acid (0.6 mmol), Cs_2CO_3 (1.5 mmol) and $Pd(PPh_3)_4$ (0.025 mmol) were added, and reaction mixture was stirred in MW reactor at 100 °C for 1 h. Reaction mixture was filtered through the pad of celite. Filtrate was diluted with EtOAc and washed with water and brine. Organic phase was separated, dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure. Crude was purified by column chromatography (gradient from Hex/EtOAc 20/1 to 8/1, v/v) to yield coupled products **6a–o**.

Ethyl 1-(2-oxo-2-phenylethyl)-3,4-diphenyl-1H-pyrazole-5carboxylate (6a). White solid, yield 84% (172 mg). $R_{\rm f} = 0.63$ (*n*-hexane/ethyl acetate 7/3, v/v), mp 112–113 °C. IR (KBr) $\nu_{\rm max}$ -cm⁻¹: 3063, 2987, 2932, 1701 (C=O), 1449, 1226, 1096, 763, 708. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 0.92 (t, J = 7.1 Hz, 3H, CH₃), 4.03 (q, J = 7.1 Hz, 2H, OCH₂), 6.13 (s, 2H, NCH₂), 7.20–7.24 (m, 3H, 3-Ph 3,4,5-H), 7.28–7.37 (m, 5H, 4-Ph 2,3,4,5,6-H), 7.38–7.43 (m, 2H, 3-Ph 2,6-H), 7.50–7.56 (m, 2H, C(O)Ph 3,5-H), 7.61–7.67 (m, 1H, C(O)Ph 4-H), 8.01–8.06 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 13.5 (CH₃), 59.2 (NCH₂), 61.0 (OCH₂), 125.2 (C-4), 127.4 (4-Ph C-4), 127.8 (3-Ph C-4), 127.9 (4-Ph C-2,6), 128.12 (3-Ph C-2,6), 128.14 (C(O)Ph C-2,6), 128.3 (3-Ph C-3,5), 129.1 (C(O)Ph C-3,4), 130.8 (4-Ph C-3,5), 131.8 (C-5), 132.5 (3Ph C-1), 133.3 (4-Ph C-1), 134.1 (C(O)Ph C-4), 134.9 (C(O)Ph C-1), 149.5 (C-3), 160.4 (COO), 192.5 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -181.7 (N-1), -67.6 (N-2). HRMS (ESI) for C₂₆H₂₂N₂NaO₃ ([M + Na]⁺): calcd *m/z* 433.1523, found *m/z* 433.1523.

Ethyl 1-[2-(4-methoxyphenyl)-2-oxoethyl]-3,4-diphenyl-1H*pyrazole-5-carboxylate (6b).* White solid, yield 74% (163 mg). $R_{\rm f} =$ 0.28 (n-hexane/ethyl acetate 4/1, v/v), mp 144-145 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3053, 2989, 2962, 2837, 1708 (C=O), 1601, 1235, 1176, 1095, 838, 699. ¹H NMR (400 MHz, $CDCl_3$) δ_H ppm: 0.91 (t, J = 6.8 Hz, 3H, CH₃), 3.90 (s, 3H, OCH₃), 3.98-4.08 (m, 2H, OCH₂), 6.09 (s, 2H, NCH₂), 6.95-7.06 (m, 2H, C(O)Ph 3,5-H), 7.18-7.25 (m, 3H, 3-Ph 3,4,5-H), 7.28-7.47 (m, 7H, 3-Ph 2,6-H; 4-Ph 2,3,4,5,6-H), 7.95-8.08 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, $CDCl_3$) δ_C ppm: 13.6 (CH₃), 55.7 (OCH₃), 58.9 (CH₂), 61.0 (OCH₂), 114.3 (C(O)Ph C-3,5), 125.1 (C-4), 127.4 (4-Ph C-4), 127.78 (3-Ph C-4), 127.86 (C(O)Ph C-1), 127.91 (4-Ph C-2,6), 128.1 (3-Ph C-2,6), 128.3 (3-Ph C-3,5), 130.5 (C(O)Ph C-2,6), 130.8 (4-Ph C-3,5), 132.0 (C-5), 132.5 (3-Ph C-1), 133.3 (4-Ph C-1), 149.3 (C-3), 160.4 (COO), 164.2 (C(O)Ph C-4), 190.8 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -181.0 (N-1), -68.5 (N-2). HRMS (ESI) for $C_{27}H_{24}N_2NaO_4$ ([M + Na]⁺): calcd m/z 463.1628, found *m*/*z* 463.1631.

1-[2-(4-fluorophenyl)-2-oxoethyl]-3,4-diphenyl-1H-Ethyl *pyrazole-5-carboxylate (6c)*. White solid, yield 97% (208 mg). $R_{\rm f} =$ 0.71 (n-hexane/ethyl acetate 7/3, v/v), mp 114-115 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3060, 2984, 2941, 1707 (C=O), 1598, 1231, 1095, 837, 699. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 0.92 (t, I = 7.1 Hz, 3H, CH₃), 4.03 (q, J = 7.1 Hz, 2H, OCH₂), 6.10 (s, 2H, NCH₂), 7.17-7.25 (m, 5H, C(O)Ph 3,5-H; 3-Ph 3,4,5-H), 7.28-7.43 (m, 7H, 3-Ph 2,6-H; 4-Ph 2,3,4,5,6-H), 8.02-8.10 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 13.5 (CH₃), 59.0 (NCH₂), 61.1 (OCH₂), 116.32 (d, ${}^{2}J_{CF} = 22.1$ Hz, C(O)Ph C-3,5), 125.2 (C-4), 127.5 (4-Ph C-4), 127.9 (3-Ph C-4), 128.0 (4-Ph C-2,6), 128.1 (3-Ph C-2,6), 128.3 (3-Ph C-3,5), 130.8 (4-Ph C-3,5), 130.85 (d, ${}^{3}J_{CF} =$ 9.7 Hz, C(O)Ph C-2,6), 131.31 (d, ${}^{4}J_{CF} = 3.0$ Hz, C(O)Ph C-1), 131.8 (C-5), 132.4 (3-Ph C-1), 133.2 (4-Ph C-1), 149.5 (C-4), 160.5 (COO), 166.33 (d, J = 256.1 Hz, C(O)Ph C-4), 190.9 (C= O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -181.8 (N-1), -67.6 (N-2). ¹⁹F NMR (376 MHz, CDCl₃) $\delta_{\rm F}$ ppm: -103.4 (C(O)Ph 4-F). HRMS (ESI) for $C_{26}H_{21}FN_2NaO_3$ ([M + Na]⁺): calcd m/z 451.1428, found *m*/*z* 451.1431.

Ethyl 1-[2-(4-chlorophenyl)-2-oxoethyl]-3,4-diphenyl-1H*pyrazole-5-carboxylate (6d).* White solid, yield 90% (200 mg). $R_{\rm f} =$ 0.74 (n-hexane/ethyl acetate 7/3, v/v), mp 135-136 °C. IR (KBr) $v_{\rm max}$, cm⁻¹: 3062, 2999 and 2992 (doublet), 2939, 1702 (C=O), 1224, 1094, 696. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 0.91 (t, *J* = 7.1 Hz, 3H, CH₃), 4.03 (q, J = 7.1 Hz, 2H, OCH₂), 6.08 (s, 2H, NCH₂), 7.19-7.24 (m, 3H, 3-Ph 3,4,5-H), 7.28-7.44 (m, 7H, 3-Ph 2,6-H; 4-Ph 2,3,4,5,6-H), 7.47-7.55 (m, 2H, C(O)Ph 3,5-H), 7.92-8.00 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 13.4 (CH₃), 58.9 (NCH₂), 60.9 (OCH₂), 125.2 (C-4), 127.3 (4-Ph C-4), 127.7 (3-Ph C-4), 127.8 (4-Ph C-2,6), 128.0 (3-Ph C-2,6), 128.2 (3-Ph C-3,5), 129.3 (C(O)Ph C-3,5), 129.4 (C(O)Ph C-2,6), 130.7 (4-Ph C-3,5), 131.6 (C-5), 132.3 (3-Ph C-1), 133.04 (C(O)Ph C-1), 133.07 (4-Ph C-1), 140.4 (C(O)Ph C-4), 149.4 (C-3), 160.3 (COO), 191.3 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) δ_N ppm: -182.0 (N-1), -67.7 (N-2). HRMS (ESI) for $C_{26}H_{21}ClN_2NaO_3$ ([M + Na]⁺): calcd m/z 467.1133, found m/z 467.1134.

1-[2-(4-hydroxyphenyl)-2-oxoethyl]-3,4-diphenyl-1H-Ethyl *pyrazole-5-carboxylate (6e)*. White solid, yield 87% (186 mg). $R_{\rm f} =$ 0.68 (n-hexane/ethyl acetate 1/1, v/v), mp 143-144 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3300 (OH), 3059, 2984, 2949, 1709 (C=O), 1603, 1234, 1170, 700. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 0.81 (t, J = 7.1 Hz, 3H, CH₃), 3.95 (q, I = 7.0 Hz, 2H, OCH₂), 6.11 (s, 2H, NCH₂), 6.88-6.99 (m, 2H, C(O)Ph 3,5-H), 7.20-7.43 (m, 10H, 3-Ph 2,3,4,5,6-H; 4-Ph 2,3,4,5,6-H), 7.94-8.01 (m, 2H, C(O)Ph 2,6-H), 10.56 (s, 1H, OH). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 13.2 (CH₃), 58.9 (NCH₂), 60.6 (OCH₂), 115.5 (C(O)Ph C-3,5), 124.1 (C-4), 125.8 (C(O)Ph C-1), 127.42 (3-Ph C-2,6; 4-Ph C-4), 127.7 (3-Ph C-4), 128.0 (4-Ph C-2,6), 128.3 (3-Ph C-3,5), 130.3 (4-Ph C-3,5), 130.7 (C(O)Ph C-2,6), 131.5 (3-Ph C-1), 132.2 (C-5), 132.8 (4-Ph C-1), 147.6 (C-3), 159.2 (COO), 162.8 (C(O)Ph C-4), 190.8 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) δ_N ppm: -177.7 (N-1), -64.9 (N-2). HRMS (ESI) for $C_{26}H_{22}N_2NaO_4$ ([M + Na]⁺): calcd *m*/*z* 449.1472, found *m*/*z* 449.1469.

3,4-bis(4-methoxyphenyl)-1-(2-oxo-2-phenylethyl)-1H-Ethyl *pyrazole-5-carboxylate (6f).* White solid, yield 77% (181 mg). $R_{\rm f} =$ 0.40 (n-hexane/ethyl acetate 4/1, v/v), mp 62-63 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 2982, 2939, 2836, 1706 (C=O), 1450, 1248, 1176, 1093, 1032, 834. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 0.97 (t, J =7.1 Hz, 3H, CH₃), 3.77 (s, 3H, 3-Ph 4-OCH₃), 3.84 (s, 3H, 4-Ph 4-OCH₃), 4.05 (q, J = 7.1 Hz, 2H, OCH₂), 6.11 (s, 2H, NCH₂), 6.72-6.80 (m, 2H, 3-Ph 3,5-H), 6.84-6.94 (m, 2H, 4-Ph 3,5-H), 7.19-7.25 (m, 2H, 4-Ph 2,6-H), 7.30-7.37 (m, 2H, 3-Ph 2,6-H), 7.48-7.57 (m, 2H, C(O)Ph 3,5-H), 7.60-7.68 (m, 1H, C(O)Ph 4-H), 7.99-8.07 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 13.7 (CH₃), 55.3 (3-Ph 4-OCH₃), 55.4 (4-Ph 4-OCH₃), 59.4 (NCH₂), 61.0 (OCH₂), 113.4 (4-Ph C-3,5), 113.7 (3-Ph C-3,5), 124.5 (C-4), 125.1 (3-Ph C-1), 125.4 (4-Ph C-1), 128.1 (C(O)Ph C-2,6), 129.1 (C(O)Ph C-3,5), 129.4 (3-Ph C-2,6), 131.8 (C-5), 132.0 (4-Ph C-2,6), 134.0 (C(O)Ph C-4), 134.9 (C(O)Ph C-1), 149.4 (C-3), 159.0 (3-Ph C-4), 159.3 (4-Ph C-4), 160.5 (COO), 192.5 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -183.6 (N-1), -71.6 (N-2). HRMS (ESI) for C₂₈H₂₆N₂NaO₅ ([M + Na]⁺): calcd m/ z 493.1734, found m/z 493.1732.

Ethyl 3,4-bis(4-methoxyphenyl)-1-[2-(4-methoxyphenyl)-2oxoethyl]-1H-pyrazole-5-carboxylate (6g). White solid, yield 91% (228 mg). $R_{\rm f} = 0.29$ (*n*-hexane/ethyl acetate 4/1, v/v), mp 67–68 ° C. IR (KBr) ν_{max} , cm⁻¹: 2938, 2837, 1707 (C=O), 1699, 1601, 1320, 1245, 1174, 1092, 1031, 835. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 0.96 (t, J = 7.1 Hz, 3H, CH₃), 3.76 (s, 3H, 4-Ph 4-OCH₃), 3.84 (s, 3H, 3-Ph 4-OCH₃), 3.90 (s, 3H, C(O)Ph 4-OCH₃), 4.05 (q, J = 7.1 Hz, 2H, OCH₂), 6.05 (s, 2H, NCH₂), 6.73-6.79 (m, 2H, 3-Ph 3,5-H), 6.85-6.91 (m, 2H, 4-Ph 3,5-H), 6.97-7.02 (m, 2H, C(O)Ph 3,5-H), 7.19-7.25 (m, 2H, 4-Ph 2,6-H), 7.30-7.36 (m, 2H, 3-Ph 2,6-H), 7.98-8.03 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) δ_C ppm: 13.6 (CH₃), 55.16 (3-Ph 4-OCH₃), 55.23 (4-Ph 4-OCH₃), 55.6 (C(O)Ph 4-OCH₃), 58.7 (NCH₂), 60.8 (OCH₂), 113.2 (4-Ph C-3,5), 113.6 (3-Ph C-3,5), 114.1 (C(O)Ph C-3,5), 124.3 (C-4), 125.2 (3-Ph C-1), 125.4 (4-Ph C-1), 127.8 (C(O)Ph C-1), 129.2 (3-Ph C-2,6), 130.3 (C(O)Ph C-2,6), 131.6 (C-5), 131.9 (4-Ph C-2,6), 149.2 (C-3), 158.8 (3-Ph C-4), 159.1 (4-Ph C-4), 160.4 (COO), 164.1 (C(O)Ph C-4), 190.8 (C=O). ¹⁵N NMR (40 MHz, CDCl₃)

 $\delta_{\rm N}$ ppm: -182.9 (N-1), -69.1 (N-2). HRMS (ESI) for $C_{29}H_{28}N_2NaO_6$ ([M + Na]⁺): calcd *m*/*z* 523.1840, found *m*/*z* 523.1840.

Ethyl 1-[2-(4-fluorophenyl)-2-oxoethyl]-3,4-bis(4-methoxyphenyl)-1H-pyrazole-5-carboxylate (6h). White solid, yield 66% (161 mg). $R_{\rm f} = 0.67$ (*n*-hexane/ethyl acetate 3/2, v/v), mp 104– 105 °C. IR (KBr) ν_{max} , cm⁻¹: 2998, 2940, 2836, 1705 (C=O), 1597, 1436, 1249, 1178, 1091, 1033, 839. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 0.84 (t, J = 7.1 Hz, 3H, CH₃), 3.71 (s, 3H, 3-Ph 4-OCH₃), 3.78 (s, 3H, 4-Ph 4-OCH₃), 3.96 (q, J = 7.1 Hz, 2H, OCH₂), 6.17 (s, 2H, NCH₂), 6.80-6.87 (m, 2H, 3-Ph 3,5-H), 6.91-6.97 (m, 2H, 4-Ph 3,5-H), 7.13-7.19 (m, 2H, 4-Ph 2,6-H), 7.23-7.30 (m, 2H, 3-Ph 2,6-H), 7.40-7.48 (m, 2H, C(O)Ph 3,5-H), 8.15-8.22 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 13.4 (CH₃), 55.1 (2 × OCH₃), 59.2 (NCH₂), 60.6 (OCH₂), 113.5 (4-Ph C-3,5), 113.8 (3-Ph C-3,5), 116.14 (d, ${}^{2}J_{CF} = 22.0$ Hz, C(O)Ph C-3,5), 123.5 (C-4), 124.71 (3-Ph C-1), 124.73 (4-Ph C-1), 128.7 (3-Ph C-2,6), 131.16, 131.20 and 131.26 (m, C-5, C(O)Ph C-1,2,6), 131.5 (4-Ph C-2,6), 147.9 (C-3), 158.6 (4-Ph 4-OCH₃), 158.9 $(3-Ph 4-OCH_3)$, 159.4 (COO), 165.56 (d, $J_{CF} = 252.9$ Hz, C(O)Ph C-4), 191.9 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm N}$ ppm: -180.0 (N-1), -66.2 (N-1). ¹⁹F NMR (376 MHz, DMSO-d₆) $\delta_{\rm F}$ ppm: -104.5 (C(O)Ph 4-F). HRMS (ESI) for C28H25FN2NaO5 ([M + Na]⁺): calcd m/z 511.1640, found m/z 511.1639.

Ethyl 1-[2-(4-chlorophenyl)-2-oxoethyl]-3,4-bis(4-methoxyphenyl)-1H-pyrazole-5-carboxylate (6i). White solid, yield 82% (207 mg). $R_{\rm f} = 0.35$ (*n*-hexane/ethyl acetate 4/1, v/v), mp 132– 133 °C. IR (KBr) ν_{max} , cm⁻¹: 2982, 2939, 2836, 1706 (C=O), 1450, 1248, 1176, 1092, 1032, 834. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 0.96 (t, J = 7.1 Hz, 3H, CH₃), 3.76 (s, 3H, 3-Ph 4-OCH₃), 3.84 (s, 3H, 4-Ph 4-OCH₃), 4.04 (q, J = 7.1 Hz, 2H, OCH₂), 6.04 (s, 2H, NCH₂), 6.72-6.81 (m, 2H, 3-Ph 3,5-H), 6.84-6.93 (m, 2H, 4-Ph 3,5-H), 7.17-7.24 (m, 2H, 4-Ph 2,6-H), 7.29-7.37 (m, 2H, 3-Ph 2,6-H), 7.45-7.53 (m, 2H, C(O)Ph 3,5-H), 7.90-8.00 (m, 2H, C(O) Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 13.6 (CH₃), 55.16 (3-Ph 4-OCH₃), 55.22 (4-Ph 4-OCH₃), 58.9 (NCH₂), 60.9 (OCH₂), 113.3 (4-Ph C-3,5), 113.6 (3-Ph C-3,5), 124.4 (C-4), 125.0 (3-Ph C-1), 125.3 (4-Ph C-1), 129.2 (3-Ph C-2,6), 129.3 (C(O)Ph C-3,5), 129.4 (C(O)Ph C-2,6), 131.4 (C-5), 131.8 (4-Ph C-2,6), 133.1 (C(O)Ph C-1), 140.4 (C(O)Ph C-4), 149.4 (C-3), 158.9 (4-Ph C-4), 159.17 (3-Ph C-4), 160.4 (COO), 191.4 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) δ_N ppm: -183.5 (N-1), -69.1 (N-2). HRMS (ESI) for $C_{28}H_{25}ClN_2NaO_5$ ([M + Na]⁺): calcd m/z 527.1344, found m/z 527.1346.

Ethyl 3-(4-fluorophenyl)-1-(2-oxo-2-phenylethyl)-4-phenyl-1Hpyrazole-5-carboxylate (6j). White solid, yield 73% (156 mg). $R_{\rm f} =$ 0.74 (*n*-hexane/ethyl acetate 7/3, v/v), mp 135–136 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3063, 2978, 2955, 1716 (C=O), 1523, 1449, 1305, 1222, 1096, 846, 768. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 0.80 (t, J = 7.1 Hz, 3H, CH₃), 3.95 (q, J = 7.0 Hz, 2H, OCH₂), 6.22 (s, 2H, NCH₂), 7.06–7.18 (m, 2H, 3-Ph 3,5-H), 7.22–7.45 (m, 7H, 3-Ph 2,6-H; 4-Ph 2,3,4,5,6-H), 7.58–7.67 (m, 2H, C(O)Ph 3,5-H), 7.70–7.79 (m, 1H, C(O)Ph 4-H), 8.05–8.16 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 13.2 (CH₃), 59.3 (NCH₂), 60.7 (OCH₂), 115.29 (d, ² $_{\rm JCF} =$ 21.5 Hz, 3-Ph C-3,5), 124.0 (C-4), 127.5 (4-Ph C-4), 128.1 (4-Ph C-3,5; C(O)Ph C-2,6), 128.62 (d, ⁴ $_{\rm JCF} =$ 3.1 Hz, 3-Ph C-4), 129.0 (C(O)Ph C-3,5), 129.43 (d, ³ $_{\rm JCF} =$ 8.2 Hz, 3-Ph C-2,6), 130.3 (4-Ph C-2,6), 131.4 (C-5), 132.5 (4-Ph C-1), 134.1 (C(O)Ph C-4), 134.3 (C(O)Ph C-1), 146.9 (C-3), 159.2 (COO), 161.72 (d, $J_{\rm CF}$ = 245.2 Hz, 3-Ph C-4), 193.0 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm N}$ ppm: -178.1 (N-1), -65.2 (N-2). ¹⁹F NMR (376 MHz, DMSO-d₆) $\delta_{\rm F}$ ppm: -114.0 (3-Ph 4-F). HRMS (ESI) for C₂₆H₂₁FN₂NaO₃ ([M + Na]⁺): calcd *m/z* 451.1428, found *m/z* 451.1427.

Ethyl 3,4-bis(4-fluorophenyl)-1-(2-oxo-2-phenylethyl)-1H*pyrazole-5-carboxylate (6k)*. White solid, yield 79% (176 mg). $R_{\rm f} =$ 0.71 (n-hexane/ethyl acetate 7/3, v/v), mp 103-104 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3063, 2976, 2949, 1704 (C=O), 1524, 1442, 1223, 1095, 856, 602. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 0.84 (t, J = 7.0 Hz, 3H, CH₃), 3.97 (q, J = 7.0 Hz, 2H, OCH₂), 6.22 (s, 2H, NCH₂), 7.10-7.38 (m, 8H, 3-Ph 2,3,5,6-H; 4-Ph 2,3,5,6-H), 7.58-7.66 (m, 2H, C(O)Ph 3,5-H), 7.71-7.78 (m, 1H, C(O)Ph 4-H), 8.06-8.13 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 13.2 (CH₃), 59.4 (NCH₂), 60.7 (NCH₂), 115.01 (d, ²J_{CF} = 21.4 Hz, 4-Ph C-3,5), 115.38 (d, ${}^{2}J_{CF} = 21.6$ Hz, 3-Ph C-3,5), 123.0 (C-4), 128.1 (C(O)Ph C-2,6), 128.47 (d, ${}^{4}J_{CF} = 3.2$ Hz, 3-Ph C-1), 128.77 (d, ${}^{4}J_{CF} = 3.2$ Hz, 4-Ph C-1), 129.0 (C(O)Ph C-3,5), 129.50 (d, ${}^{3}J_{CF} = 8.3$ Hz, 3-Ph C-2,6), 131.5 (C-5), 132.40 (d, ${}^{3}J_{CF} = 8.2 \text{ Hz}, 4\text{-Ph C-2,6} \text{ 134.1 (C(O)Ph C-1), 134.3 (C(O)Ph C-4),}$ 147.1 (C-3), 159.1 (COO), 161.67 (d, $J_{CF} = 244.1$ Hz, 4-Ph C-4), 161.76 (d, $J_{CF} = 245.2$ Hz, 3-Ph C-4), 193.0 (C=O). ¹⁵N NMR $(40 \text{ MHz}, \text{DMSO-d}_6) \delta_N \text{ ppm}: -177.8 \text{ (N-1)}, -65.1 \text{ (N-2)}.$ ¹⁹F NMR $(376 \text{ MHz}, \text{DMSO-d}_6) \delta_F$ ppm: -114.7 (4-F), -113.9 (4-F). HRMS (ESI) for $C_{26}H_{20}F_2N_2NaO_3$ ([M + Na]⁺): calcd m/z 469.1334, found m/z 469.1334.

Ethyl 3-(4-fluorophenyl)-4-(4-methoxyphenyl)-1-(2-oxo-2-phenylethyl)-1H-pyrazole-5-carboxylate (6l). White solid, yield 80% (183 mg). $R_{\rm f} = 0.68$ (*n*-hexane/ethyl acetate 3/2, v/v), mp 87–88 °C. IR (KBr) ν_{max} , cm⁻¹: 2984, 2941, 2837, 1724, 1702 (C=O), 1440, 1223, 1176, 1087, 832. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 0.97 $(t, J = 7.1 \text{ Hz}, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.06 (q, J = 7.1 \text{ Hz}, 3H, CH_3)$ 2H, OCH₂), 6.11 (s, 2H, NCH₂), 6.86-6.96 (m, 4H, 3-Ph 3,5-H; 4-Ph 3,5-H), 7.18–7.24 (m, 2H, 4-Ph 2,6-H), 7.35–7.41 (m, 2H, 3-Ph 2,6-H), 7.50-7.57 (m, 2H, C(O)Ph 3,5-H), 7.61-7.68 (m, 1H, C(O) Ph 4-H), 7.98-8.08 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 13.7 (CH₃), 55.4 (OCH₃), 59.2 (NCH₂), 61.1 (OCH_2) , 113.5 (4-*Pc* C-3,5), 115.26 (d, ${}^2J_{CF} = 21.5$ Hz, 3-Ph C-3,5), 124.7 (C-4), 125.0 (4-Ph C-1), 128.1 (C(O)Ph C-2,6), 128.67 (d, ⁴J_{CF} = 3.2 Hz, 3-Ph C-1), 129.1 (C(O)Ph C-3,5), 129.88 (d, ${}^{3}J_{CF} =$ 8.1 Hz, 3-Ph C-2,6), 131.88 (C-5), 131.91 (4-Ph C-2,6), 134.1 (C(O) Ph C-4), 134.8 (C(O)Ph C-1), 148.7 (C-3), 159.1 (4-Ph C-4), 160.4 (COO), 162.55 (d, $J_{CF} = 247.1$ Hz, 3-Ph C-4), 192.4 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -182.2 (N-1), -70.0 (N-2). ¹⁹F NMR (376 MHz, CDCl₃) δ_F ppm: -114.2 (3-Ph 4-F). HRMS (ESI) for $C_{27}H_{23}FN_2NaO_4$ ([M + Na]⁺): calcd m/z 481.1534, found m/z481.1535.

Ethyl 3-(4-fluorophenyl)-1-[2-(4-fluorophenyl)-2-oxoethyl]-4phenyl-1H-pyrazole-5-carboxylate (6m). White solid, yield 76% (170 mg). $R_{\rm f} = 0.64$ (*n*-hexane/ethyl acetate 3/2, v/v), mp 104– 105 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3059, 2999, 2962, 1711 (C=O), 1599, 1232, 1156, 1095, 840. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 0.80 (t, J = 7.1 Hz, 3H, CH₃), 3.95 (q, J = 7.1 Hz, 2H, OCH₂), 6.21 (s, 2H, NCH₂), 7.07–7.17 (m, 2H, 3-Ph 3,5-H), 7.21– 7.51 (m, 9H, 3-Ph 2,6-H; 4-Ph 2,3,4,5,6-H; C(O)Ph 3,5-H), 8.16– 8.22 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) $\delta_{\rm C}$ ppm: 13.2 (CH₃), 59.3 (NCH₂), 60.7 (OCH₂), 115.29 (d, ²J_{CF} = 21.6 Hz, 3-Ph C-3,5), 116.12 (d, ²J_{CF} = 22.0 Hz, C(O)Ph C-3,5), 124.0 (C-4), 127.5 (4-Ph C-4), 128.1 (4-Ph C-3,5), 128.60 (d, ⁴J_{CF} = 3.1 Hz, 3-Ph C-1), 129.42 (d, ⁴J_{CF} = 8.3 Hz, 3-Ph C-2,6), 130.3 (4-Ph C-2,6), 131.10 (d, ⁴J_{CF} = 2.8 Hz, C(O)Ph C-1), 131.21 (d, ³J_{CF} = 9.6 Hz, C(O)Ph C-2,6), 131.4 (C-5), 132.5 (4-Ph C-1), 147.0 (C-3), 159.2 (COO), 161.72 (d, J_{CF} = 245.1 Hz, 3-Ph C-4), 165.54 (d, J_{CF} = 252.9 Hz, C(O)Ph C-4), 191.7 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm F}$ ppm: -114.0 (4-F), -104.4 (4-F). HRMS (ESI) for C₂₆H₂₀F₂N₂NaO₃ ([M + Na]⁺): calcd *m*/*z* 469.1334, found *m*/*z* 469.1332.

Ethyl 3-(4-fluorophenyl)-1-[2-(4-fluorophenyl)-2-oxoethyl]-4-(4methoxyphenyl)-1H-pyrazole-5-carboxylate (6n). White solid, yield 79% (188 mg). $R_{\rm f} = 0.69$ (*n*-hexane/ethyl acetate x/x, v/v), mp 81–82 °C. IR (KBr) ν_{max} , cm⁻¹: 2993, 2956, 2840, 1706 (C= O), 1597, 1440, 1232, 1093, 844. ¹H NMR (400 MHz, CDCl₃) $\delta_{\rm H}$ ppm: 0.97 (t, J = 7.1 Hz, 3H, CH₃), 3.85 (s, 3H, OCH₃), 4.05 (q, J = 7.1 Hz, 2H, OCH₂), 6.07 (s, 2H, NCH₂), 6.86–6.96 (m, 4H, 4-Ph 3,5-H; C(O)Ph 3,5-H), 7.16-7.24 (m, 4H, 3-Ph 3,5-H; 4-Ph 2,6-H), 7.33-7.42 (m, 2H, 3-Ph 2,6-H), 8.01-8.11 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, CDCl₃) $\delta_{\rm C}$ ppm: 13.6 (CH₃), 55.3 (OCH₃), 58.9 (NCH₂), 61.0 (OCH₂), 113.4 (4-Ph C-3,5), 115.16 (d, ${}^{2}J_{CF} =$ 21.5 Hz, C(O)Ph C-3,5), 116.23 (d, ${}^{2}J_{CF} = 22.0$ Hz, 3-Ph C-3,5), 124.6 (C-4), 124.9 (4-Ph C-1), 128.51 (d, ${}^{4}J_{CF} = 3.3$ Hz, 3-Ph C-1), 129.75 (d, ${}^{3}J_{CF} = 8.1$ Hz, 3-Ph C-2,6), 130.73 (d, ${}^{3}J_{CF} =$ 9.5 Hz, C(O)Ph C-2,6), 131.15 (d, ${}^{4}J_{CF} = 3.1$ Hz, C(O)Ph C-1), 131.7 (C-5), 131.8 (4-Ph C-2,6), 148.7 (C-3), 159.0 (4-Ph C-4), 160.3 (COO), 162.45 (d, $J_{CF} = 247.1$ Hz, 3-Ph C-4), 166.24 (d, $J_{\rm CF} = 256.1$ Hz, C(O)Ph C-4), 190.8 (C=O). ¹⁵N NMR (40 MHz, CDCl₃) $\delta_{\rm N}$ ppm: -182.8 (N-1), -69.2 (N-2). ¹⁹F NMR (376 MHz, $CDCl_3$) δ_F ppm: -114.2 (4-F), -103.3 (4-F). HRMS (ESI) for $C_{27}H_{22}F_2N_2NaO_4$ ([M + Na]⁺): calcd m/z 499.1440, found m/z 499.1439.

Ethyl 3,4-bis(4-fluorophenyl)-1-[2-(4-fluorophenyl)-2-oxoethyl]-1H-pyrazole-5-carboxylate (60). White solid, yield 85% (197 mg). $R_{\rm f} = 0.62$ (*n*-hexane/ethyl acetate 7/3, v/v), mp 105–106 °C. IR (KBr) ν_{max} , cm⁻¹: 3000, 2984, 2946, 1710 (C=O), 1599, 1444, 1231, 840. ¹H NMR (400 MHz, DMSO-d₆) $\delta_{\rm H}$ ppm: 0.84 (t, J =7.1 Hz, 3H, CH_3), 3.97 (q, J = 7.0 Hz, 2H, OCH_2), 6.22 (s, 2H, NCH₂), 7.09-7.38 (m, 8H, 3-Ph 2,3,5,6-H; 3-Ph 2,3,5,6-H), 7.40-7.51 (m, 2H, C(O)Ph 3,5-H), 8.12–8.25 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, DMSO-d₆) δ_C ppm: 13.2 (CH₃), 59.3 (NCH₂), 60.8 (OCH₂), 115.02 (d, ${}^{2}J_{CF} = 21.4$ Hz, 4-Ph C-3,5), 115.39 (d, ${}^{2}J_{CF} = 21.5$ Hz, 3-Ph C-3,5), 116.13 (d, ${}^{2}J_{CF} = 22.0$ Hz, C(O)Ph C-3,5), 123.0 (C-4), 128.45 (d, ${}^{4}J_{CF} =$ 3.2 Hz, 3-Ph C-1), 128.75 (d, ${}^{4}J_{CF} = 3.3$ Hz, 4-Ph C-1), 129.50 (d, ${}^{3}J_{CF} = 8.3$ Hz, 3-Ph C-2,6), 131.09 (d, ${}^{4}J_{CF} = 2.7$ Hz, C(O)Ph C-1), 131.21 (d, ${}^{3}J_{CF} = 9.6$ Hz, C(O)Ph C-2,6), 131.5 (C-5), 132.40 (d, ${}^{3}J_{CF} = 8.3$ Hz, 4-Ph C-2,6), 147.1 (C-3), 159.1 (COO), 161.67 (d, $J_{CF} = 244.1$ Hz, 4-Ph C-4), 161.77 (d, J_{CF} = 245.3 Hz, 3-Ph C-4), 165.55 (d, J_{CF} = 253.0 Hz, C(O)Ph C-4), 191.69 (C=O). ¹⁵N NMR (40 MHz, DMSO-d₆) $\delta_{\rm N}$ ppm: -177.7 (N-1), -65.1 (N-2). ¹⁹F NMR (376 MHz, DMSO d_6) δ_F ppm: -114.7 (4-F), -113.9 (4-F), -104.4 (4-F). HRMS (ESI) for $C_{26}H_{19}F_3N_2NaO_3$ ([M + Na]⁺): calcd m/z 487.1240, found m/z487.1238.

General procedure for synthesis of methyl 3,4-diaryl-1-(2aryl-2-oxoethyl)-1*H*-pyrazole-5-carboxylates (7a–c). An appropriate pyrazole derivative 6a–c (0.25 mmol) was dissolved in MeOH (0.05 M), K₂CO₃ (0.125 mmol) was added, and mixture was refluxed for 3 h. After full conversion solvent was partially evaporated, residue was diluted with EtOAc and washed with water and brine. Organic phase was separated, dried over anhydrous Na₂SO₄, filtered off and concentrated under reduced pressure. Crude was purified by column chromatography (Hex/ EtOAc 20/1, v/v) to yield products 7a–c.

1-(2-oxo-2-phenylethyl)-3,4-diphenyl-1H-pyrazole-5-Methyl carboxylate (7a). White solid, yield 94% (93 mg). $R_{\rm f} = 0.63$ (nhexane/ethyl acetate 7/3, v/v), mp 138–139 °C. IR (KBr) ν_{max} ,cm⁻¹: 3059, 2976, 2939, 1716 (C=O), 1451, 1355, 1319, 1231, 1100, 774 and 757 (doublet), 705 and 691 (doublet). ¹H NMR (400 MHz, acetone-d₆) $\delta_{\rm H}$ ppm: 3.53 (s, 3H, CH₃), 6.24 (s, 2H, CH₂), 7.19-7.45 (m, 10H, 3-Ph 2,3,4,5,6-H; 4-Ph 2,3,4,5,6-H), 7.59-7.68 (m, 2H, C(O)Ph 3,5-H), 7.70-7.78 (m, 1H, C(O)Ph 4-H), 8.12-8.19 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, acetoned₆) δ_C ppm: 52.0 (NCH₂), 60.1 (OCH₂), 125.4 (C-4), 128.2 (4-Ph C-4), 128.5 (3-Ph C-4), 128.7 (4-Ph C-2,6), 128.8 (3-Ph 2,6-H), 128.9 (3-Ph 3,5-H; C(O)Ph C-2,6), 129.8 (C(O)Ph C-3,5), 131.4 (4-Ph C-3,5), 132.4 (C-5), 133.7 (3-Ph C-1), 134.1 (4-Ph C-1), 134.8 (C(O)Ph C-4), 135.9 (C(O)Ph C-1), 149.5 (C-3), 161.2 (COO), 193.4 (C=O). ¹⁵N NMR (40 MHz, acetone-d₆) $\delta_{\rm N}$ ppm: -178.7 (N-1), -63.3 (N-2). HRMS (ESI) for $C_{25}H_{20}N_2NaO_3$ ([M + Na]⁺): calcd m/z419.1366, found *m*/*z* 419.1367.

1-[2-(4-methoxyphenyl)-2-oxoethyl]-3,4-diphenyl-1H-Methyl *pyrazole-5-carboxylate (7b).* White solid, yield 92% (98 mg). $R_{\rm f} =$ 0.49 (n-hexane/ethyl acetate 7/3, v/v), mp 163-164 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3025, 2962, 2836, 1712 (C=O), 1601, 1237, 1176, 1096, 698. ¹H NMR (400 MHz, acetone- d_6) δ_H ppm: 3.53 (s, 3H, COOCH₃), 3.94 (s, 3H, OCH₃), 6.17 (s, 2H, CH₂), 7.09-7.17 (m, 2H, C(O)Ph 3,5-H), 7.20-7.43 (m, 10H, 3-Ph 2,3,4,5,6-H; 4-Ph 2,3,4,5,6-H), 8.09-8.17 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, acetone-d₆) $\delta_{\rm C}$ ppm: 51.9 (COOCH₃), 56.1 (OCH₃), 59.8 (CH₂), 115.0 (C(O)Ph C-3,5), 125.3 (C-4), 128.2 (4-Ph C-4), 128.4 (3-Ph C-4), 128.68 (4-Ph C-2,6), 128.72 (C(O)Ph C-1), 128.8 (3-Ph C-2,6), 128.9 (3-Ph C-3,5), 131.2 (C(O)Ph C-2,6), 131.4 (4-Ph C-3,5), 132.5 (C-5), 133.7 (3-Ph C-1), 134.2 (4-Ph C-1), 149.4 (C-3), 161.2 (COO), 165.2 (C(O)Ph C-4), 191.5 (C=O). ¹⁵N NMR (40 MHz, acetone-d₆) δ_N ppm: -178.2 (N-1), -63.5 (N-2). HRMS (ESI) for $C_{26}H_{22}N_2NaO_4$ ([M + Na]⁺): calcd m/z 449.1472, found *m*/*z* 449.1470.

Methyl 1-[2-(4-fluorophenyl)-2-oxoethyl]-3,4-diphenyl-1Hpyrazole-5-carboxylate (7c). White solid, yield 96% (100 mg). $R_{\rm f} =$ 0.66 (*n*-hexane/ethyl acetate 7/3, v/v), mp 114–115 °C. IR (KBr) $\nu_{\rm max}$, cm⁻¹: 3061, 2929, 1720 (C=O), 1596, 1232, 1086, 841, 700. ¹H NMR (400 MHz, acetone-d₆) $\delta_{\rm H}$ ppm: 3.56 (s, 3H, CH₃), 6.23 (s, 2H, CH₂), 7.13–7.20 (m, 2H, C(O)Ph 3,5-H), 7.23–7.29 (m, 3H, 3-Ph 3,4,5-H), 7.31–7.41 (m, 4H, 3-Ph 2,6-H; 4-Ph 2,6-H), 7.59– 7.66 (m, 2H, 4-Ph 3, 5H), 7.70–7.77 (m, 1H, 4-Ph 4-H), 8.12–8.18 (m, 2H, C(O)Ph 2,6-H). ¹³C NMR (101 MHz, acetone-d₆) $\delta_{\rm C}$ ppm: 52.0 (CH₃), 60.2 (CH₂), 115.64 (d, ²J_{CF} = 21.6 Hz, C(O)Ph C-3,5), 124.3 (C-4), 128.6 (4-Ph C-4), 128.7 (3-Ph C-4), 128.9 (3-Ph C-3,5), 129.0 (4-Ph C-2,6), 129.8 (3-Ph C-2,6), 130.29 (d, ⁴J_{CF} = 3.4 Hz, C(O)Ph C-1), 132.4 (4-Ph C-3,5), 133.39 (d, ${}^{3}J_{\rm CF} = 8.2$ Hz, C(O)Ph C-2,6), 133.5 (3-Ph C-1), 134.8 (4-Ph C-1), 135.9 (C-5), 149.6 (C-3), 161.1 (COO), 163.13 (d, $J_{\rm CF} = 244.5$ Hz, C(O)Ph C-4), 193.3 (C=O). 15 N NMR (40 MHz, acetone-d₆) $\delta_{\rm N}$ ppm: -178.3 (N-1), -63.2 (N-2). 19 F NMR (376 MHz, acetone-d₆) $\delta_{\rm F}$ ppm: -116.5 (C(O)Ph 4-F). HRMS (ESI) for C₂₅H₁₉FN₂NaO₃ ([M + Na]⁺): calcd *m/z* 437.1272, found *m/z* 437.1269.

Determination of HPLC-log P

HPLC-log *P* values were determined using Shimadzu HPLC system equipped with apHera C18 column (10 × 6 mm, 5 µm, Supelco, Bellefonte, PA, USA). Each sample was dissolved in the internal standard mixture, consisting of triphenylene (99.9%, Carl Roth) and toluene (≥98%, Sigma-Aldrich). Analysis was performed at 1.5 mL min⁻¹ flow rate in the linear gradient where mobile phase consisted of methanol and 0.01 M phosphate buffer (pH 7.4). The HPLC-log *P* values were calculated from the measured retention times using previously published equation.⁵⁷

Plasma protein binding (PPB)

Retention times of the analytes were measured with Shimadzu HPLC system on the CHIRALPAK®HAS stationary phase (50×3 mm, 5 µm, Chiral Technologies, DAICEL Group, Europe SAS, France). The mobile phase A consisted of 50 mM aqueous ammonium acetate buffer (pH 7.4) and phase B of 2-propanol according to Valko et al.65 Analysis was performed at prolonged 1 mL min⁻¹ flow rate in the linear gradient. Retention capacity factors (k') were calculated by using DMSO or a substance with 0% HAS binding for systems' dead time (R_{t_0}) . The system was calibrated by injecting the reference compounds: acetylsalicylic acid (CAS 69-72-7), betamethasone (CAS 378-44-9), budesonide (CAS 5133-22-3), carbamazepine (CAS 298-46-4), cimetidine (CAS 51481-61-9), ciprofloxacin (CAS 85721-33-1), indomethacin (CAS 53-86-1), isoniazid (CAS 54-85-3), metronidazole (CAS 443-48-1), nicardipine (CAS 55985-32-5), nizatidine (CAS 76963-41-2) and warfarin (CAS 81-81-2) obtained from Sigma-Aldrich, diclofenac (CAS 15307-86-5) from EMD Chemicals Inc., flumazenil (CAS 78755-81-4) from ABX and ketoprofen (CAS 22071-15-4) from LKT Labs. The logarithmic capacity factors of the references' Rt $(\log(k'))$ on the HSA column were plotted against the %PPB values from literature. The slope and the intercept were used to convert the log(k') of the compounds (6a, c, f, h, mo) to %PPB using the regression equation.⁶⁶

Biological evaluation

Cell cultures. SW480 (human colon adenocarcinoma, adherent, epithelial, ATCC number: CCL-228), HT29 (human colon adenocarcinoma, adherent, epithelial, ATCC number: HTB-38), and HCT116 (human colon adenocarcinoma, adherent, epithelial-like, ATCC number: CCL-247) cells were maintained in RPMI-1640 medium (Sigma-Aldrich). Medium was supplemented with heat-inactivated FBS (Biowest), penicillin–streptomycin (Sigma; with 10 000 units penicillin and 10 mg streptomycin per mL in 0.9% NaCl) and L-glutamine (Sigma; 200 mM solution). Cell cultures were cultivated at 37 °C,

maintaining a humidified atmosphere consisting of 5% CO₂. GibcoTM trypsin–EDTA (0.05%) was used for cell passage.

Cell viability (MTT assay). To evaluate cell viability, SW480, HT29, and HCT116 cells were harvested from culture flasks by trypsinization and seeded into 96-well plates in 4000 cells per well, 3000 cells per well and 4000 cells per well densities, respectively. After 24 h preincubation period, cells were treated in triplicates with nine different doses of each compound (6a-o and 7a-c) for 96 h. After treatment compound solutions were replaced with MTT solution (MTT reagent in PBS, 5 mg L^{-1}) diluted in a 6:1 ratio in non-supplemented RPMI-1640 medium and were additionally incubated for 2 h. After incubation, the medium was removed, and formazan product was dissolved in DMSO. Optical densities were measured at 550 nm with TECAN Infinite® M200 PRO microplate reader using a reference wavelength of 690 nm to correct unspecific absorption. The quantity of viable cells was normalized to untreated controls. The GI₅₀ values were calculated from dose-response curves.

Viability staining *via* Calcein AM/Hoechst/PI. SW480, HT29 and HCT116 cells were seeded in 24-well plates in a cell density of 6000 cells per well and settled for 24 h. Afterwards cells were treated with 4 μ M of test compounds **6a**, **c**, **f**, **h**, **m–o** for 96 h. Cells were stained with propidium iodide (Sigma Aldrich, final concentration 2 μ g mL⁻¹) and Hoechst 33342 (ThermoFisher Scientific, final concentration 0.5 μ M) for 60 min and with Calcein AM (Merck, BioReagent, final concentration 50 μ M) for 15 min, washed two times with PBS and finally covered with PBS for further analysis. Fluorescence microscopic evaluation was performed on a Evos FL Cell Imaging System (ThermoFisher Scientific).

Fluorescence-activated cell sorting (FACS). HCT116 cells (40 000 cells per well) were treated with the test compounds (6c, 6m) for 72 h in the concentration range of 5, 10 and 20 μ M. After treatment cells were washed with DPBS (Sigma; modified, without calcium chloride and magnesium chloride) and trypsinized using a trypsin–EDTA solution (GibcoTM, 0.05%). After additional washing with DPBS, cells were stained at +4 °C overnight using PI/HFS solution (50 μ g mL⁻¹).⁶⁷ Samples were then analysed using Guava® easyCyteTM 8HT (Merck Millipore) flow cytometer with Guava Clean 3.1 software. The amount of debris was in the range of 9.2 \pm 2.0%.

Conclusions

Proceeding from the natural marine alkaloid lamellarin O, a scaffold hopping from a central pyrrole to pyrazole resulted in 18 fully characterized derivatives. Structure–activity relationships revealed the importance of a fluorine in the *para*-position of the phenyl substituent in phenyl-2-oxoethyl scaffold. The most cytotoxic compounds inhibited cell proliferation in the low micromolar range in three colorectal cancer cell lines, namely HCT116, HT29, and SW480. The similarity of the investigated GI_{50} values demonstrated the absence of conventional resistance mechanism between the cell lines. Pronounced effects on the cell cycle were observed resulting in G1 and predominantly G2/M phase arrest. This set of lamellarin O inspired pyrazole derivatives is an important entry for natural product-derived drug candidates.

Author contributions

Conceptualization, E. A., A. Ž. and V. P.; methodology, E. A., A. Ž. and V. P.; formal analysis, K. D., N. F. E. S.-B.; investigation, K. D., N. F., E. S.-B., V. P.; resources, A. Š., E. A. and V. P.; data curation, E. A. and V. P.; writing—original draft preparation, K. D.; writing—review and editing, E. A., A. Ž., A. Š. and V. P.; visualization, K. D. and V. P.; supervision, E. A., A. Ž. and V. P.; funding acquisition, V. P. and E. A. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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References

- 1 J. Bracegirdle, L. P. Robertson, P. A. Hume, M. J. Page, A. v. Sharrock, D. F. Ackerley, A. R. Carroll and R. A. Keyzers, *J. Nat. Prod.*, 2019, **82**, 2000–2008.
- 2 H. Zhang, M. M. Conte, X. C. Huang, Z. Khalil and R. J. Capon, *Org. Biomol. Chem.*, 2012, **10**, 2656–2663.
- 3 H. Fan, J. Peng, M. T. Hamann and J. F. Hu, *Chem. Rev.*, 2008, **108**, 264–287.
- 4 T. Fukuda, F. Ishibashi and M. Iwao, *Alkaloids: Chemistry and Biology*, Elsevier Inc., 1st edn, 2020, vol. 83, pp. 1–112, ISSN: 1099-4831.
- 5 C. Bailly, Curr. Med. Chem.: Anti-Cancer Agents, 2004, 4, 363–378.
- 6 C. Bailly, Mar. Drugs, 2015, 13, 1105-1123.
- 7 D. Pla, F. Albericio and M. Álvarez, *MedChemComm*, 2011, 2, 689–697.
- 8 P. Krishnaiah, V. L. N. Reddy, G. Venkataramana, K. Ravinder, M. Srinivasulu, T. V. Raju, K. Ravikumar, D. Chandrasekar, S. Ramakrishna and Y. Venkateswarlu, *J. Nat. Prod.*, 2004, 67, 1168–1171.
- 9 D. Matulja, F. Vranješević, M. Kolympadi Markovic, S. K. Pavelić and D. Marković, *Molecules*, 2022, 27, 1449, DOI: 10.3390/molecules27041449.
- 10 F. Plisson, X. Huang, H. Zhang, Z. Khalil and R. J. Capon, *Chem.-Asian J.*, 2012, 7, 1616–1623.
- P. Sopha, N. Phutubtim, B. Chantrathonkul, P. Ploypradith, S. Ruchirawat and M. Chittchang, *Toxicology*, 2021, 462, 152963, DOI: 10.1016/j.tox.2021.152963.

- 12 S. Khiati, Y. Seol, K. Agama, I. D. Rosa, S. Agrawal, K. Fesen, H. Zhang, K. C. Neuman and Y. Pommier, *Mol. Pharmacol.*, 2014, 86, 193–199.
- C. Ballot, A. Martoriati, M. Jendoubi, S. Buche, P. Formstecher, L. Mortier, J. Kluza and P. Marchetti, *Marine Drugs*, 2014, 12, 779–798.
- 14 C. Ballot, J. Kluza, S. Lancel, A. Martoriati, S. M. Hassoun, L. Mortier, J. C. Vienne, G. Briand, P. Formstecher, C. Bailly, R. Nevière and P. Marchetti, *Apoptosis*, 2010, 15, 769–781.
- 15 C. Ballot, J. Kluza, A. Martoriati, U. Nyman, P. Formstecher, B. Joseph, C. Bailly and P. Marchetti, *Mol. Cancer Ther.*, 2009, 8, 3307–3317.
- 16 X. C. Huang, X. Xiao, Y. K. Zhang, T. Talele, A. Salim, Z. S. Chen and R. Capon, *Mar. Drugs*, 2014, **12**, 3818–3837.
- 17 I. Satyanarayana, D. Y. Yang and T. J. Liou, *RSC Adv.*, 2020, **10**, 43168–43174.
- 18 Q. Zhang, Y. Feng and D. Kennedy, Cell. Mol. Life Sci., 2017, 74, 777–801.
- 19 V. Kumar, A. Awasthi, A. Salam and T. Khan, *J. Org. Chem.*, 2019, **84**, 11596–11603.
- 20 D. Imbri, J. Tauber and T. Opatz, *Mar. Drugs*, 2014, **12**, 6142–6177.
- 21 D. Morikawa, K. Morii, Y. Yasuda, A. Mori and K. Okano, *J. Org. Chem.*, 2020, **85**, 8603–8617.
- 22 V. Colligs, S. P. Hansen, D. Imbri, E. J. Seo, O. Kadioglu, T. Efferth and T. Opatz, *Bioorg. Med. Chem.*, 2017, 25, 6137–6148.
- 23 K. Klumthong, P. Chalermsub, P. Sopha, S. Ruchirawat and P. Ploypradith, *J. Org. Chem.*, 2021, **86**, 14883–14902.
- 24 T. Fukuda, Y. Nanjo, M. Fujimoto, K. Yoshida, Y. Natsui, F. Ishibashi, F. Okazaki, H. To and M. Iwao, *Bioorg. Med. Chem.*, 2019, 27, 265–277.
- 25 L. Zheng, T. Gao, Z. Ge, Z. Ma, J. Xu, W. Ding and L. Shen, *Eur. J. Med. Chem.*, 2021, 214, 113226, DOI: 10.1016/ j.ejmech.2021.113226.
- 26 T. Fukuda, T. Umeki, K. Tokushima, G. Xiang, Y. Yoshida, F. Ishibashi, Y. Oku, N. Nishiya, Y. Uehara and M. Iwao, *Bioorg. Med. Chem.*, 2017, 25, 6563–6580.
- 27 T. Fukuda, M. Anzai, A. Nakahara, K. Yamashita, K. Matsukura, F. Ishibashi, Y. Oku, N. Nishiya, Y. Uehara and M. Iwao, *Bioorg. Med. Chem.*, 2021, 34, 116039, DOI: 10.1016/j.bmc.2021.116039.
- 28 K. Klumthong, P. Chalermsub, P. Sopha, S. Ruchirawat and P. Ploypradith, *J. Org. Chem.*, 2021, **86**, 14883–14902.
- 29 P. K. Mykhailiuk, Chem. Rev., 2021, 121, 1670-1715.
- 30 W. Byon, S. Garonzik, R. A. Boyd and C. E. Frost, *Clin. Pharmacokinet.*, 2019, **58**, 1265–1279.
- 31 X. Li, Y. Yu and Z. Tu, *Molecules*, 2021, 26, 1202, DOI: 10.3390/molecules26051202.
- 32 P. R. Lazzara and T. W. Moore, *RSC Med. Chem.*, 2020, **11**, 18–29.
- 33 V. Milišiūnaitė, A. Kadlecová, A. Žukauskaitė, K. Doležal, M. Strnad, J. Voller, E. Arbačiauskienė, W. Holzer and A. Šačkus, *Mol. Diversity*, 2020, 24, 1025–1042.

- 34 V. Milišiūnaitė, R. Paulavičiūtė, E. Arbačiauskienė, V. Martynaitis, W. Holzer and A. Šačkus, *Beilstein J. Org. Chem.*, 2019, **15**, 679–684.
- 35 V. Milišiūnaitė, E. Plytninkienė, R. Bakšienė, A. Bieliauskas,
 S. Krikštolaitytė, G. Račkauskienė, E. Arbačiauskienė and
 A. Šačkus, *Molecules*, 2021, 26, 5604, DOI: 10.3390/ molecules26185604.
- 36 B. Razmienė, E. Řezníčková, V. Dambrauskienė, R. Ostruszka, M. Kubala, A. Žukauskaitė, V. Kryštof, A. Šačkus and E. Arbačiauskienė, *Molecules*, 2021, 26, 6747, DOI: 10.3390/molecules26216747.
- 37 G. Matulevičiūtė, E. Arbačiauskienė, N. Kleizienė,
 V. Kederienė, G. Ragaitė, M. Dagilienė, A. Bieliauskas,
 V. Milišiūnaitė, F. A. Sløk and A. Šačkus, *Molecules*, 2021,
 26, 3808, DOI: 10.3390/molecules26133808.
- 38 V. Milišiūnaitė, E. Arbačiauskienė, E. Řezníčková, R. Jorda, V. Malínková, A. Žukauskaitė, W. Holzer, A. Šačkus and V. Kryštof, *Eur. J. Med. Chem.*, 2018, **150**, 908–919.
- 39 B. Razmienė, V. Vojáčková, E. Řezníčková, L. Malina, V. Dambrauskienė, M. Kubala, R. Bajgar, H. Kolářová, A. Žukauskaitė, E. Arbačiauskienė, A. Šačkus and V. Kryštof, *Bioorg. Chem.*, 2022, **119**, 105570, DOI: **10.1016**/ j.bioorg.2021.105570.
- 40 G. Varvuolytė, L. Malina, A. Bieliauskas, B. Hošíková, H. Simerská, H. Kolářová, N. Kleizienė, V. Kryštof, A. Šačkus and A. Žukauskaitė, *Dyes Pigm.*, 2020, 183, 108666, DOI: 10.1016/j.dyepig.2020.108666.
- 41 E. Arbačiauskienė, V. Laukaitytė, W. Holzer and A. Šačkus, *Eur. J. Org. Chem.*, 2015, 2015, 5663–5670.
- 42 Y. Wu, C. Tang, R. Rui, L. Yang, W. Ding, J. Wang, Y. Li, C. C. Lai, Y. Wang, R. Luo, W. Xiao, H. Zhang, Y. Zheng and Y. He, *Acta Pharm. Sin. B*, 2020, **10**, 512–528.
- 43 B. Xiong, S. Chen, P. Zhu, M. Huang, W. Gao, R. Zhu, J. Qian, Y. Peng, Y. Zhang, H. Dai and Y. Ling, *Med. Chem.*, 2019, 15, 743–754.
- 44 M. A. Düfert, K. L. Billingsley and S. L. Buchwald, J. Am. Chem. Soc., 2013, 135, 12877–12885.
- 45 A. Secrieru, P. M. O'Neill and M. L. S. Cristiano, *Molecules*, 2019, 25, 42, DOI: 10.3390/molecules25010042.
- 46 R. Lin, G. Chiu, Y. Yu, P. J. Connolly, S. Li, Y. Lu, M. Adams,
 A. R. Fuentes-Pesquera, S. L. Emanuel and
 L. M. Greenberger, *Bioorg. Med. Chem. Lett.*, 2007, 17, 4557–4561.
- 47 M. Guerrero, J. Pérez, J. Ros, V. Branchadell, E. Pellicer, J. Sort and J. Pons, *Curr. Org. Synth.*, 2013, **11**, 149–155.
- 48 M. Iškauskienė, G. Ragaitė, F. A. Sløk and A. Šačkus, *Mol. Diversity*, 2020, 24, 1235–1251.
- 49 K. Dzedulionytė, M. Veikšaitė, V. Morávek,
 V. Malinauskienė, G. Račkauskienė, A. Šačkus,
 A. Žukauskaitė and E. Arbačiauskienė, *Molecules*, 2022, 27, 8666, DOI: 10.3390/molecules27248666.
- 50 C. F. R. A. C. Lima, A. S. M. C. Rodrigues, V. L. M. Silva, A. M. S. Silva and L. M. N. B. F. Santos, *ChemCatChem*, 2014, 6, 1291–1302.
- 51 J. Sherwood, J. H. Clark, I. J. S. Fairlamb and J. M. Slattery, *Green Chem.*, 2019, **21**, 2164–2213.

- 52 S. Bhat K, V. Lanke, J. D. Prasad and K. R. Prabhu, *Appl. Catal.*, *A*, 2020, **596**, 117516, DOI: **10.1016**/ j.apcata.2020.117516.
- 53 K. S. M. Salih and Y. Baqi, *Catalysts*, 2020, 10, 4, DOI: 10.3390/catal10010004.
- 54 Z. Li, C. Liu, W. Shi, X. Cai, Y. Dai, C. Liao, W. Huang and H. Qian, *Bioorg. Med. Chem. Lett.*, 2018, **26**, 703–711.
- 55 C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 2012, **64**, 4–17.
- 56 D. Camp, A. Garavelas and M. Campitelli, *J. Nat. Prod.*, 2015, **78**, 1370–1382.
- 57 C. Vraka, L. Nics, K. H. Wagner, M. Hacker, W. Wadsak and M. Mitterhauser, *Nucl. Med. Biol.*, 2017, **50**, 1–10.
- 58 G. M. Nitulescu, *Molecules*, 2022, 27, 3300, DOI: 10.3390/ molecules27103300.
- 59 C. A. Lipinski, Adv. Drug Delivery Rev., 2016, 101, 34-41.
- 60 B. M. Johnson, Y.-Z. Shu, X. Zhuo and N. A. Meanwell, *J. Med. Chem.*, 2020, **63**, 6315–6386.

- 61 L. C. Crowley, B. J. Marfell and N. J. Waterhouse, *Cold Spring Harbor Protocols*, 2016, **2016**, 778–781.
- 62 C. Ballot, A. Martoriati, M. Jendoubi, S. Buche, P. Formstecher, L. Mortier, J. Kluza and P. Marchetti, *Mar. Drugs*, 2014, **12**, 779–798.
- 63 S. G. Summerfield, J. W. T. Yates and D. A. Fairman, *Pharm. Res.*, 2022, **39**, 213–222.
- 64 X. Liu, M. Wright and C. E. C. A. Hop, *J. Med. Chem.*, 2014, 57, 8238–8248.
- 65 K. Valko, S. Nunhuck, C. Bevan, M. H. Abraham and D. P. Reynolds, *J. Pharm. Sci.*, 2003, **92**, 2236–2248.
- 66 C. Vraka, S. Mijailovic, V. Fröhlich, M. Zeilinger, E. M. Klebermass, W. Wadsak, K. H. Wagner, M. Hacker and M. Mitterhauser, *Nucl. Med. Biol.*, 2018, 58, 20–32.
- 67 I. Nicoletti, G. Migliorati, M. C. Pagliacci, F. Grignani and C. Riccardi, J. Immunol. Methods, 1991, 139, 271–279.