Konstantinos M. Kasiotis*, Evangelia N. Tzanetou, Dimitrios Stagos, Nikolas Fokialakis, Eleni Koutsotheodorou, Dimitrios Kouretas and Serkos A. Haroutounian*

Novel conformationally constrained pyrazole derivatives as potential anti-cancer agents

DOI 10.1515/znb-2015-0053
Received March 26, 2015; accepted April 30, 2015

Abstract: The synthesis of 17 novel conformationally constrained pyrazole derivatives is reported herein, along with the assessment of their anti-proliferative and anti-angiogenic activities. The evaluation of their inhibitory effect on cell proliferation against HepG2, HeLa, and MCF-7 cells revealed the pyrrolo[2,3-*g*]indazole **23** as a potent inhibitor of cell growth with IC_{50} values of 5 μM. Additionally, the inhibition of vascular endothelial growth factor by pyrazoles **20** and **23** (30 % and 35 %, respectively) in HeLa supernatant cells was evidenced. These findings highlight the usefulness of these compounds as potential scaffolds for the design and development of novel anti-cancer agents with pronounced anti-angiogenic activity.

Keywords: angiogenesis; anti-cancer agents; anti-proliferative; pyrazoles; synthesis.

***Corresponding authors: Konstantinos M. Kasiotis,** Laboratory of Pesticides Toxicology, Benaki Phytopathological Institute, Department of Pesticides Control and Phytopharmacy, 8 St. Delta Street, Athens, Kifissia 14561, Greece, Tel.: +30-210-8180357, Fax: +30-210-8180223, E-mail: k.kasiotis@outlook.com; and **Serkos A. Haroutounian,** Department of Animal Sciences and Aquaculture, Agricultural University of Athens, Iera odos 75, Athens 11855, Greece, Tel.: +30-210-5294247, Fax: +30-210-5294265, E-mail: sehar@aua.gr
Evangelia N. Tzanetou: Department of Animal Sciences and Aquaculture, Agricultural University of Athens, Iera odos 75, Athens 11855, Greece
Dimitrios Stagos, Eleni Koutsotheodorou and Dimitrios Kouretas: Department of Biochemistry and Biotechnology, University of Thessaly, 26 Ploutonos Street, 41221 Larissa, Greece
Nikolas Fokialakis: Faculty of Pharmacy, Department of Pharmacognosy and Natural Products Chemistry, University of Athens, Panepistimiopolis, Zografou 15771, Athens, Greece

1 Introduction

Cancer represents a major public health concern that accounts as the second most common cause of death in the USA and elsewhere, causing nearly one of every four deaths. The American Cancer Society estimates that during 2014 in the USA 1 665 540 new cancer cases will be diagnosed and 585 720 cancer deaths will occur [1].

The main characteristic of cancer consists in the loss of control of cell growth, which eventually leads to the formation of tumors that frequently become life-threatening by obstructing vessels or organs. However, death is most usually provoked by the spread of the primary tumor to one or several sites in the body through the metastasis process, which makes surgical intervention almost impossible. Other types of cancers known as leukemias involve the build-up of a vast number of white cells in the blood. In an attempt to treat cancer, various molecules have been screened for the selective target of cancer cells and the minimization of the side effects to healthy organism compartments.

Azaheterocycles comprise an important class of compounds that exhibit diverse biological activities, including the battle against cancer as well. In particular, numerous synthetic small molecules from various groups of azaheterocycles have been reported to influence carcinogenesis and are currently in clinical trials [2, 3] or approved for the treatment of various cancer types [3, 4]. Indicatively, carbazoles possessing a unique tricyclic ring system have been permitted for the treatment of cancer [5], while phenazines [6] and several piperazines [7] have been found to display IC_{50} values in the nanomolar range against quinone reductase inhibitors. Recently, azoaryl sulfides were proved as selective anti-breast cancer agents with IC_{50} values in the low micromolar range [8, 9]. In this respect, pyrazoles that constitute a prominent family among azaheterocycles comprise a class of molecules with a prominent position in the anti-cancer portfolio as having been evidenced in numerous publications [10].

Consequently, the design and synthesis of novel pyrazoles has become the subject of several research groups. The synthesis of anti-cancer pyrazoles was recently reviewed by Kumari et al. [11], indicating that several analogues are in preclinical or initial-phase clinical trials. In the course of our longstanding interest concerning the development of novel bioactive heterocycles [12, 13], we have designed and synthesized a sequence of novel bioactive molecules containing the pyrazole residue [12–14]. Herein, we present the outcome of our vision to design and synthesize several novel molecules containing the pyrazole structural backbone fused with commercially available constrained ketone substrates. Our final goal was the assessment of their anti-proliferative and anti-angiogenic profiles.

In this context, the initial design of the target compounds was based on reports establishing that ring systems containing the (*E*)-2-benzylidene-1-tetralones and (*E*)-2-benzylidene-1-tetralone backbone exhibit remarkable cytotoxic activity [15], initiating the use of α-tetralones as precursors for the synthesis of bioactive compounds [16, 17]. In another application, 6-methoxytetralone has been utilized as a substrate for the construction of steroids [18]. It is noteworthy that 2-(1*H*-pyrazol-1-yl)-thiazole derivatives, which possess a conformationally constrained scaffold, similar to the one envisaged in this work, exhibited a potent EP1 receptor antagonist profile [19]. Additionally, a noticeable number of indanone derivatives constitute lead compounds for the treatment of Alzheimer's disease [20]. Therefore, it is apparent from bibliography that fused pyrazoles can display pronounced biological activities, and their further exploitation should be a constant goal of medicinal chemists. The potential bioactivity of the targeted compounds was preliminarily assessed by the Cheminformatics software MOLINSPIRATION [21]. The latter has been used in numerous publications as a preliminary tool that can assist rational chemical design (see [22]). The bibliographic results and the preliminary bioactivity potential revealed by Cheminformatics prompted us to design and synthesize molecules that combine the indanone/tetralone and indolone moieties with the pyrazole core. Thus, we herein present the substrate-dependent/directed synthesis of various series of novel pyrazole derivatives using as starting materials three commercially available fused ketones abiding two similar synthetic routes. Some of the final products displayed anti-proliferative activity and possible anti-angiogenic action. Additional exploitation of these compounds might provide agents with therapeutic activity against various cancer malignancies.

2 Results and discussion

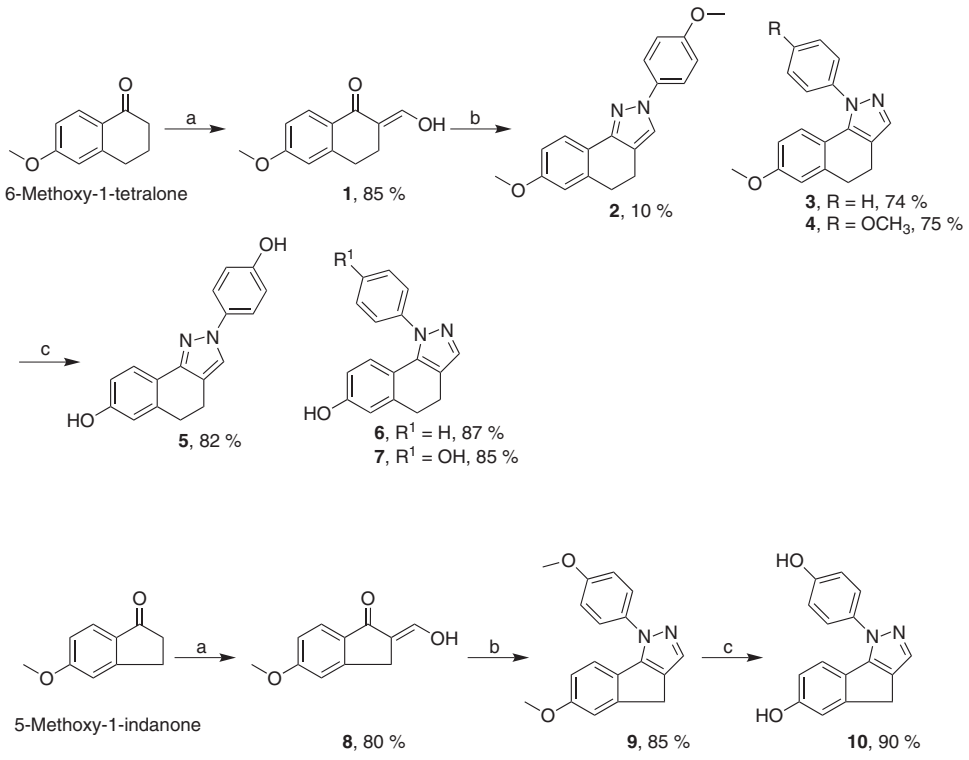
2.1 Chemical synthesis

2.1.1 First synthetic part

A general route to access the target pyrazole derivatives includes the α-acylation of the corresponding 3,4-dihydronaphthalenon-1-one substrate and the subsequent condensation with a phenylhydrazine derivative. In general, the reaction of a substituted β-dicarbonyl derivative with a molecule of hydrazine is expected to produce a mixture of two isomeric pyrazoles. It must be pointed out, however, that the two reactive centers of the hydrazine molecule may potentially react with the corresponding reaction sites of the dicarbonyl compounds tautomers, revealing the possible formation of eight possible isomers. Since the outcome of the aforementioned reaction depends greatly on the nature of both diketone substrate and the acylating agent, we were prompted to investigate the two possible distinct pathways thoroughly.

The first pathway involves the formylation of 6-methoxy-1-tetralone and 5-methoxy-1-indanone to afford the hydroxymethylene derivatives **1** and **8** (Scheme 1). Double condensation of the latter with phenyl- (or 4-methoxyphenyl-) hydrazine furnished predominantly the pyrazoles **4**, **3**, **9**, while only a small amount (10 %) of the regioisomeric pyrazole **2** was obtained (when substrate **1** was used). The regioselectivity of this condensation may be rationalized considering that the formyl carbons of compounds **1** and **8** react predominantly with the terminal-most nucleophilic NH_2 of phenylhydrazines to form an imine intermediate. The latter by intramolecular ring closure provided the corresponding benzo[*g*]indazole derivatives. Subsequently, the demethylation of compounds **2**, **4**, **3**, **9** afforded the desired phenolic pyrazoles **5**, **7**, **6**, **10**.

The structure of the pyrazole products was elucidated based on 2D-nuclear overhauser effect (NOE) NMR spectroscopy experiments. More specifically, the assigned stereochemistry of compounds **4**, **3**, **9** is consistent with the observed strong cross peak between 9-H (and 8-H for compound **9)** and Ar–H (for compounds **2** and **4** see correlations in Fig. 1). On the contrary, for compound **2** the observed enhancement of the aromatic protons Ar–H corresponds to 3-H. These results were further confirmed by gradient inverse detected long-range $^{1}H–^{15}N$ correlation experiments [23]. In this regard, for compound **2**, a four-bond correlation was observed between the aromatic proton 9-H (7.92 ppm) and the deshielded nitrogen atom (N-1) that resonates at δ = 285.2 ppm. It must also be



Scheme 1: Reagents and conditions: (a) NaH, HCOOEt, DMF, THF; (b) $RC_6H_4NHNH_2{\cdot}HCl$, DMF/THF 3:1, 120 °C; (c) BBr_3, CH_2Cl_2, –78 °C.

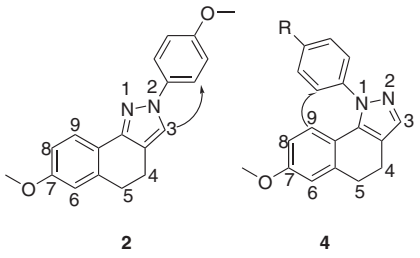
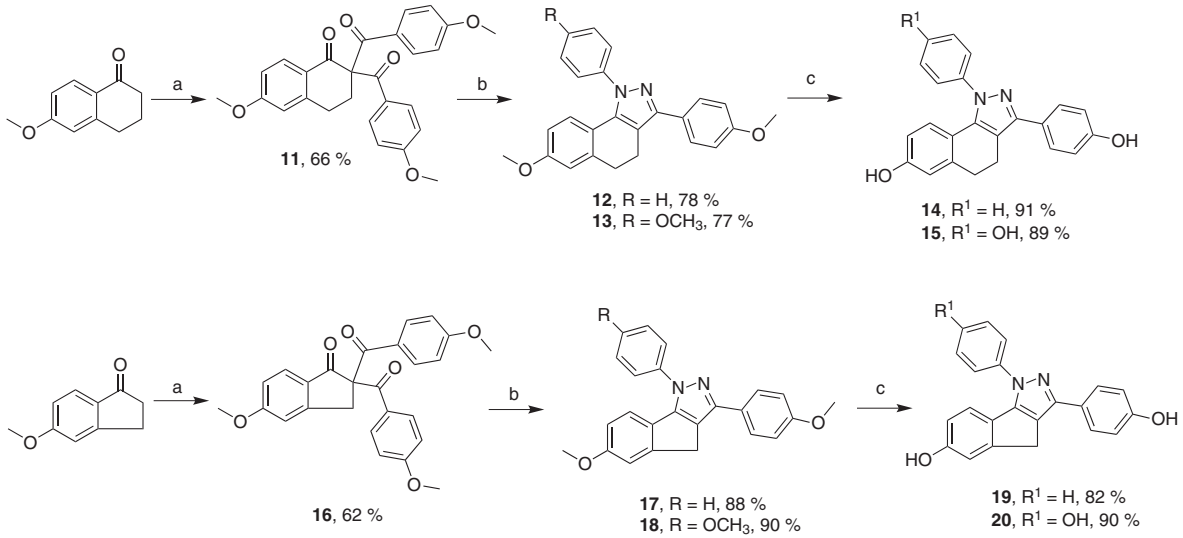


Fig. 1: NOESY correlations for compounds **2** and **4**.

noted that according to the allocated structure the other nitrogen atom (N-2) resonates at δ = 212.2 ppm and correlates with the *N*-attached aromatic protons. For compound **4**, a four-bond correlation was observed between the pyrazole proton 3-H (7.52 ppm) and the nitrogen atom that resonates at δ = 205.4 ppm. The latter corresponds to the shielded N-1 that also correlates with the *N*-attached aromatic protons. Correlation between the deshielded nitrogen atom N-2 (resonating at 305.3 ppm) with the aliphatic protons 4-H (2.97 ppm) was not observed. In the case of condensation of formate **1** with phenylhydrazine hydrochloride, we encountered only the pyrazole **3** whose conformation (same as the one of **4**) was again confirmed by nuclear overhauser effect spectroscopy (NOESY) and long-range ^1H–^{15}N experiments.

The second pathway involves the synthesis of triaryl substituted pyrazoles (Scheme 2). In this respect, the Li enolates of the commercially available 6-methoxy-1-tetralone and 5-methoxy-1-indanone (obtained by treatment with lithium bis(trimethylsilyl)amide [LiHMDS]) were regioselectively acylated with *p*-anisoyl chloride to afford triketones **11** and **16** surprisingly. The use of limited amounts of base (up to 0.5 equivalents) in order to avoid this overacylation was unsuccessful, since we were not able to obtain any amount of diketone. The change of solvent and use of toluene, as it was adapted in the second synthetic part (presented below), did not furnish the diketone, and surprisingly led to a decreased yield. Nevertheless, the desired novel sterically constrained pyrazoles were approached smoothly using the procedure described by Stauffer and colleagues in 2000 [24].

In particular, the condensation of triketones (**11** and **16**) with *N*-substituted hydrazine hydrochlorides in refluxing DMF–THF (3:1) afforded respectively the benzo[*g*]indazoles **12**, **13** and the indeno[1,2-*c*]pyrazoles **7**, **8** with yields ranging from 74 % to 90 %. Subsequent ether cleavage using BBr_3 in CH_2Cl_2 at –78 °C provided the desired diphenols **14**, **19** and triphenols **15**, **20** (yields 82 %–91 %) as white crystalline solids. The configuration of these novel pyrazoles was elucidated, through 2D-NOE NMR spectroscopy experiments (Fig. 2). More specifically,



Scheme 2: Reagents and conditions: (a) LHMDS, $CH_3C_6H_4COCl$, THF; (b) $RC_6H_4NHNH_2{\cdot}HCl$, DMF/THF 3:1, 120 °C; (c) BBr_3, CH_2Cl_2, –78 °C.

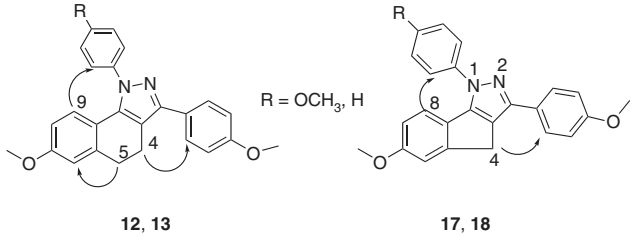


Fig. 2: NOESY correlations for benzo[*g*]indazoles and indeno[1,2-*c*] pyrazoles.

the assigned stereochemistry of compounds **17** and **18** is consistent with the observed strong cross peak between 8-H and Ar–H. For compounds **12** and **13**, the strongest observed enhancement with the aromatic protons Ar–H (of the C5 substituted ring) corresponds to the 4-H of the tetralone moiety. These results were additionally confirmed by gradient inverse detected long-range ^1H–^{15}N correlation experiments.

In this regard, for compound **12** a four-bond correlation was observed between the aromatic proton 9-H (6.76 ppm) and the shielded nitrogen atom that resonates at δ = 203.6 ppm. Furthermore, based on the assigned structure, the other nitrogen atom resonates at δ = 299.7 ppm and correlates with the *N*-attached aromatic protons; however, its signal is not intense (the ^1H–^{15}N gradient heteronuclear multiple quantum correlation [GHMQC] NMR spectrum is presented as Supplementary Information available online). The conformation of compound **17** was confirmed in a similar manner by long-range ^1H–^{15}N experiments.

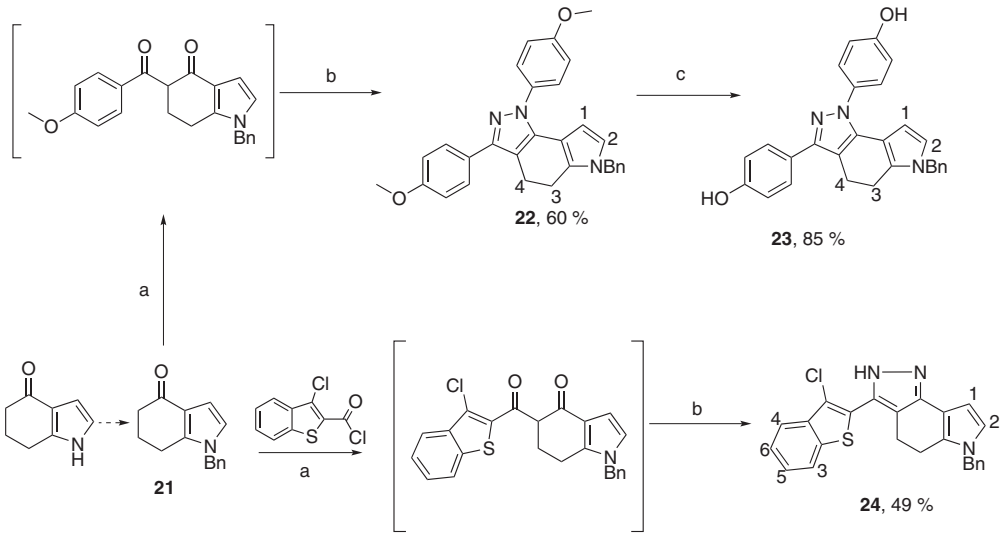
2.1.2 Second synthetic part

To extend the use of ketone substrates, the *N*-benzylated indolone **21** was selected as starting material for the synthesis of novel indazole derivatives using a modification of a procedure published previously by our group [12]. Briefly, indolone in the presence of lithium hexamethyldisilazane (LHMDS) base under anhydrous conditions provided the corresponding anion, which was subsequently condensed with *p*-anisoyl chloride or 3-chlorobenzothiophene-2-carbonyl chloride to yield the 1,3-diketone derivatives. The in situ condensation of the latter with hydrazine and its methoxyphenyl counterpart gave in one step the desired pyrazole derivatives (compounds **24** and **22**) in moderate yields (Scheme 3). The latter was attributed to the competing imine formation reaction of non-acylated indolone. Finally, deprotection of indazole **22** provided the diphenolic compound **23**.

2.2 Cytostatic activities

2.2.1 Assessment of inhibition of cancer and endothelial cell proliferation

The anti-carcinogenic activities of novel pyrazole derivatives were tested against hepatocellular (HepG2), cervix (HeLa), and breast (MCF-7) cancer cell lines. The assessed IC_{50} values for each cell line are depicted in Table 1. Three of compounds tested were determined as the most active showing a dose-dependent inhibition of the growth of all three cancer cell lines according to the



Scheme 3: Reagents and conditions: (a) LHMDS, $CH_3OC_6H_4COCl$, or $C_9H_4Cl_2OS$, toluene; (b) $CH_3OC_6H_4NHNH_2{\cdot}HCl$, or NH_2NH_2, AcOH, reflux; (c) BBr_3, CH_2Cl_2, –78 °C.

Table 1: Cytostatic activities of synthesized compounds in endothelial and tumor cell lines

Compound	IC_{50} (μM)[a,b]		
	HepG2	HeLa	MCF-7
5	17.0 ± 5.1	22.9 ± 7.2	47.3 ± 13.8
6	88.5 ± 11.8	> 100	31.1 ± 9.1
7	34 ± 8.8	17 ± 5.8	21 ± 4.9
10	64.7 ± 11.5	90.0 ± 16.9	60.3 ± 9.4
14	70.4 ± 12.1	64.5 ± 4.8	82.9 ± 14.4
15	81.6 ± 11.1	63.3 ± 12.7	75.7 ± 9.3
17	13.4 ± 4.3	42.1 ± 8.5	24.4 ± 5.9
18	50.1 ± 10.6	28.1 ± 4.9	33.4 ± 10.1
19	20.8 ± 5.1	50.4 ± 13.7	19.2 ± 6.2
20	19.8 ± 7.2	5.8 ± 3.8	13.4 ± 3.6
22	33.2 ± 6.5	12.9 ± 3.2	19.8 ± 5.9
23	3.0 ± 1.1	3.3 ± 0.9	5.0 ± 2.1
24	7.1 ± 3.2	6.9 ± 2.4	12.2 ± 5.0

[a]IC_{50}, concentration of the compound that reduces cell proliferation by 50%.[b]Number of independent experiments (n = 3).

2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilide (XTT) cell proliferation assay (Fig. 3). Compound **23** was determined as the most active since it inhibited the growth of HepG2 cells by 93 % at 20 μM and MCF-7 cell growth by 48 %, 87 %, and 91 % at 20, 40, and 60 μM, respectively (Fig. 3a and d). Moreover, compound **24** exhibited statistically significant inhibitory activity against HepG2 and MCF-7 cell growth at 20, 60, and 120 μM by 39 %, 50 %, and 98 %, and 34 %, 48 %, and 54 %, respectively (Fig. 3b and e). Finally, the triphenol substituted pyrazole **20** displayed on HeLa and MCF-7 cells IC_{50} comparable values to those of compounds **23** and **24**. Overall, compound **23** exhibited the most potent inhibitory activity against the growth of all tested cancer cell lines with IC_{50} values of 3, 3.3, and 5 μM against HepG2, HeLa, and MCF-7 cells, respectively.

2.2.2 Assessment of anti-angiogenic activity

2.2.2.1 In vitro anti-angiogenic effects using the tube formation assay

In order to reveal the mode of anti-cancer properties for the most potent among novel pyrazoles, we assessed their in vitro anti-angiogenic properties using the tube formation assay in human microvascular endothelial cells (HMEC-1). Among the compounds tested, pyrazoles **20**, **23**, and **24** were determined the most potent in inhibiting the tube formation at concentrations of 30 and 100 μM and suggesting their potent anti-angiogenic properties (Table 2).

2.2.2.2 Evaluation of novel pyrazole effects against the VEGF expression

Tumor angiogenesis starts when cancerous tumor cells release molecules sending signals to surrounding normal host tissue and activating certain genes in the host tissue, which initiates the growth of new blood vessels. Thus, in order to reveal the molecular mechanism of the anti-angiogenic activity of novel pyrazoles, their effects on the vascular endothelial growth factor (VEGF)

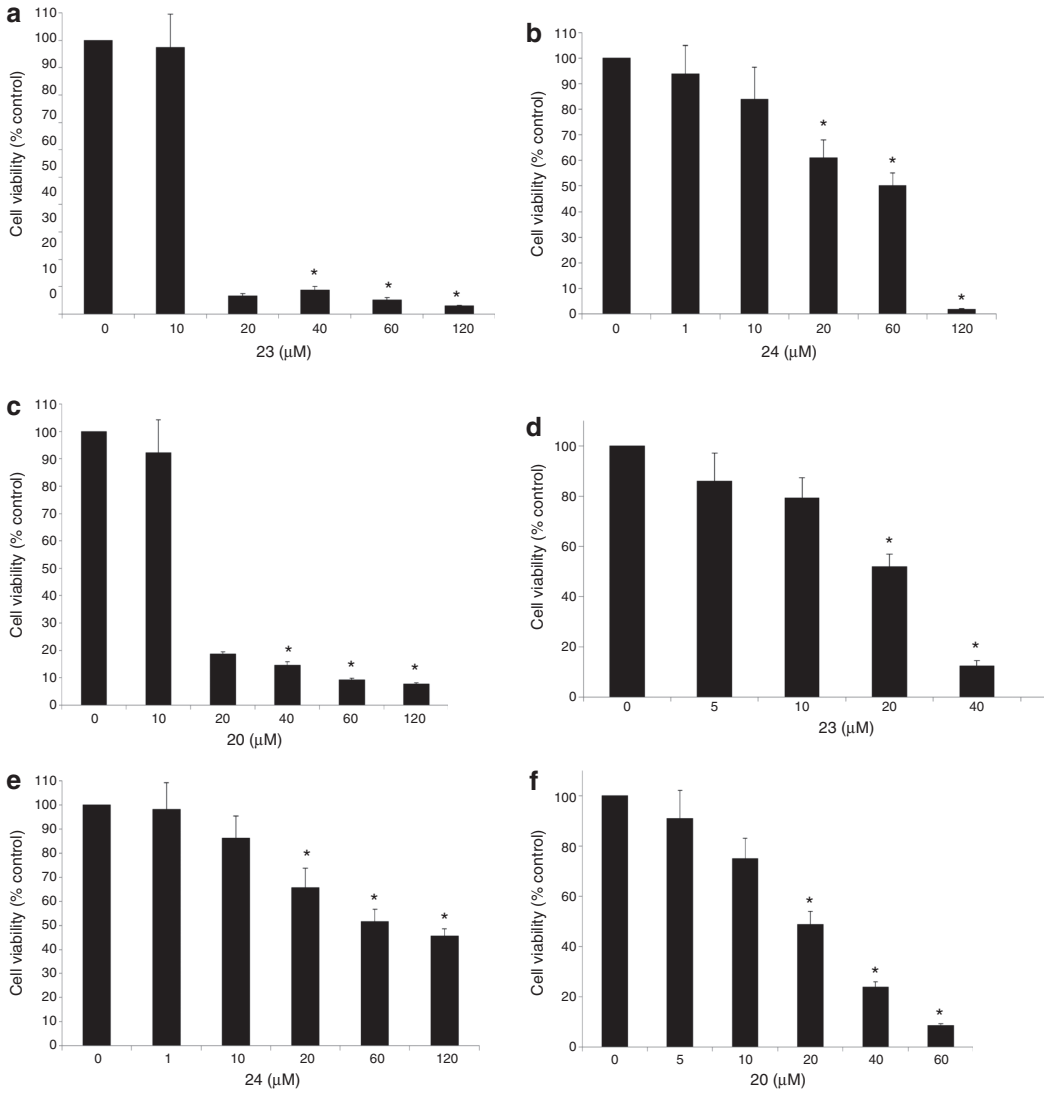


Fig. 3: Effects of compounds **20**, **23**, and **24** on (a), (b), and (c) HepG2 cell growth, and on (d), (e), and (f) MCF-7 cell growth. Adherent cells proliferated in 96-well plates (10^4 cells per well) were incubated with different concentrations of the tested compounds for 24 h. Cell proliferation was determined by XTT assay. Results were expressed as a percent of cell proliferation of control. The data shown are the mean ± SD from three independent experiments carried out in triplicate; *$p < 0.05$ vs. control.

Table 2: Assessment of in vitro anti-angiogenic effects of heterocyclic derivatives using tube formation assay in HMEC-1.

Compounds	30 μM	100 μM
20	2.5[a]	2[a]
23	2.5[a]	2[a]
24	2[a]	1.5[a]

[a]Score 0, complete inhibition – no tubes; Score 1, few tubes; Score 2, inhibition; Score 3, no inhibition, number of experiments ($n = 3$).

expression – the most important pro-angiogenic molecule – were determined in HeLa cancer cells. The respective results showed that among the tested compounds, pyrazoles **20**, **23**, and **24** inhibited significantly the VEGF levels in supernatant HeLa cells cultures (Fig. 4). Compounds **20** and **23** were the most active, since they inhibited by 30 % and 35 %, respectively, the VEGF levels at 20 μM concentration.

2.3 Cheminformatics and Bioactivity

The Cheminformatics conducted by MOLINSPIRATION predicted that compound **23** was the most active, amongst compounds synthesized, exhibiting activity as a G-protein-coupled receptor (GPCR) ligand, kinase inhibitor (KI), nuclear receptor ligand (NRL), and enzyme inhibitor (EI)

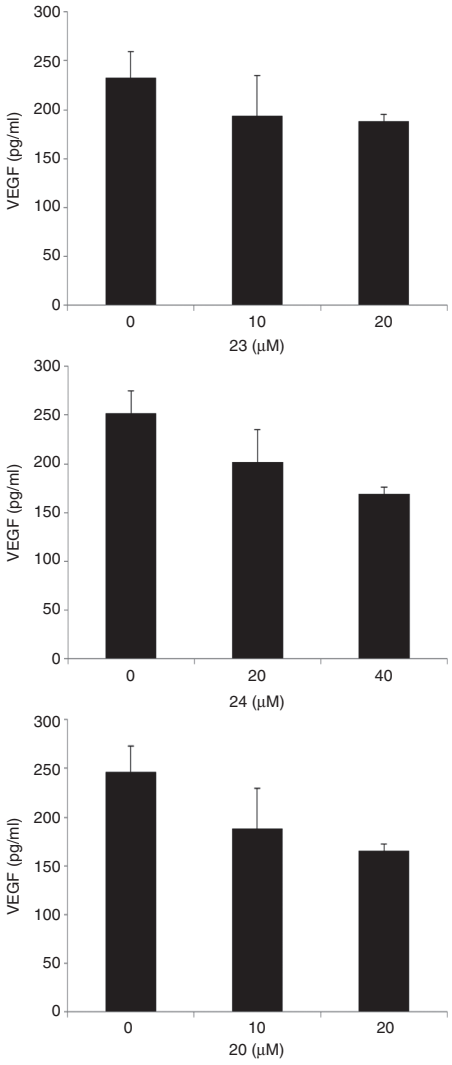


Fig. 4: Effects of heterocyclic derivatives **20**, **23**, and **24** on VEGF expression in HeLa cells. Media conditioned by incubation with HeLa cultures were evaluated for VEGF protein secretion by ELISA. The data shown were the mean ± SD from three independent experiments carried out in duplicate; *$p < 0.05$ vs. control.

(Table 3). The majority of presented compounds showed KI activity, with compounds **10, 19** and **20** projecting as the most efficient KIs (Table 3). Compound **24** that inhibited tube formation at concentrations of 30 μM exhibited only substantial KI in MOLINSPIRATION. The prognostic comparison of five- with six-membered cores (as pairs e.g. **7**–**10**, **14**–**19**, **15**–**20**) showed that five-membered scaffolds are better KIs while six-membered ones are superior NRLs. According to this approach, methoxy derivatives demonstrate lower activity than their hydroxylated analogues. Surprisingly, and in contrast with the Cheminformatics prediction, compounds **5** and **7** did not differ in activity, indicating that their configuration does not interplay significantly in the bioactivity. Overall, the comparison of all presented compounds with compound **23** proposes that the pronounced activity of **23** might be primarily attributed to its superordinate performance in terms of enzyme inhibition, combined with the relative high values of GPCR and NRL. Future molecular modeling studies, especially for the diphenol **23** and pyrrolo[2,3-*g*]indazole **24**, will reveal the structure activity relationship modes, and assist the structural modifications that will enhance the cytostatic and anti-angiogenic activities.

3 Conclusions

Novel conformationally rigid pyrazole derivatives with potential cytostatic activity have been prepared following an efficient synthetic protocol. A diphenolic substituted pyrazole **23** had the most potent inhibitory activity against the growth of the panel of tested cancer cell lines with IC_{50} values of 3, 3.3, and 5 μM in HepG2, HeLa, and MCF-7 cells, respectively. These results render it as a potential anticancer scaffold. As regards the anti-angiogenic activity both in the tube formation assay in HMEC-1 and the inhibition of VEGF, a mixed activity profile of compounds was evidenced. Compound **24** was the most active in the tube formation assay and compounds **20** and **23** exhibited anti-angiogenesis action in the VEGF expression.

To further exploit these structures, in future endeavors, targeted molecular modeling and virtual screening approaches will be pursued in order to propose the synthesis of additional derivatives that might possess enhanced anti-cancer activities. The latter is an immediate future project of our group, devoted primarily to the enhancement of the activity of the promising indazole diphenol **23** and to a lesser extent pyrrolo[2,3-*g*]indazole **24** by molecular modeling guided chemical modifications.

4 Experimental section

4.1 General information

All anhydrous reactions were carried out under argon atmosphere. Solvents were dried by distillation prior to use. Solvent mixtures employed in chromatography were reported as volume-to-volume ratios. Starting materials were purchased from Sigma-Aldrich (analytical reagent grades; St. Louis, USA) and used without further

Table 3: Prediction of bioactivity of compounds by MOLINSPIRATION (Cheminformatics) [21].

Compound	GPCR	ICM	KI	NRL	PI	EI
23	0.27	−0.12	0.23	0.38	−0.31	0.26
24	0.04	−0.34	0.29	0.05	−0.38	0.06
22	0.19	−0.22	0.16	0.25	−0.34	0.17
20	0.19	−0.03	0.42	0.10	−0.15	0.05
19	0.20	−0.03	0.43	0.10	−0.16	0.05
18	0.09	−0.13	0.31	−0.03	−0.20	−0.04
17	0.10	−0.14	0.33	−0.04	−0.21	−0.05
15	0.22	−0.10	0.18	0.29	−0.28	0.06
14	0.23	−0.11	0.18	0.30	−0.29	0.06
13	0.12	−0.19	0.09	0.14	−0.31	−0.03
12	0.14	−0.21	0.10	0.14	−0.33	−0.03
10	0.21	0.02	0.44	0.14	−0.33	0.11
9	0.14	−0.12	0.36	0.02	−0.31	−0.01
7	0.29	−0.13	0.30	0.27	−0.49	0.09
6	0.27	−0.13	0.28	0.23	−0.56	0.09
5	0.08	−0.03	0.04	0.04	−0.51	−0.06
4	0.20	−0.25	0.22	0.12	−0.47	−0.03
3	0.18	−0.27	0.20	0.08	−0.58	−0.04
2	0.01	−0.17	−0.02	−0.08	−0.48	−0.17

GPCR, G-protein-coupled receptor ligand; ICM, ion channel modulator; KI, kinase inhibitor; NRL, nuclear receptor ligand; PI, protease inhibitor; EI, enzyme inhibitor.

purification. Analytical thin-layer chromatography (TLC) was conducted on Merck glass plates (Darmstadt, Germany) coated with silica gel 60 F_{254} and spots were visualized with UV light or/and an alcohol solution of anisaldehyde. Flash column chromatography was performed using Merck silica gel 60 (230–400 mesh ASTM).

Melting points were determined on a Büchi melting point apparatus (BÜCHI Labortechnik AG, Flawil, Switzerland) and are uncorrected. ^{1}H and 2D NMR spectra were recorded at 400 MHz on a Bruker DRX-400 spectrometer (Billerica, MA, USA) in the indicated solvents. The coupling constants are recorded in Hertz (Hz) and the chemical shifts are reported in parts per million (ppm; δ scale) downfield from tetramethylsilane, which was used as an internal standard (by asterisk are indicated the overlapped peaks). Infrared spectra were obtained on a Nicolet Magna 750 (GMI-Inc, Ramsey, MN, USA), series II spectrometer.

For the ^{1}H–^{15}N GHMQC spectrum, data were acquired as 3072 × 400 data points with a total of 290 transients accumulated/t_1 increment. Pulse widths were 8.55 μs for ^{1}H and 27.7 μs for ^{15}N at powers of 0 and –3 dB. The F1 spectral window employed was set from 100 to 400 ppm. Pulsed field gradients, gt1–gt3, had durations of 0.8 ms. Gradient pairs were optimized as 70:30:50 for ^{15}N.

4.2 HPLC purification

When required, compounds were purified by semi-preparative high-performance liquid chromatography (HPLC) [column: Kromasil 100-5, C-18, (25 cm × 10 mm); mobile phase CH_3CN–H_2O (8:2); detector: UV (λ = 300 nm); flow: 1.6 mL min^{-1}; load: 5 mg per 100 μL of solution in the mobile phase]. The HPLC system was a Hewlett Packard 1100 series instrument (Agilent Technologies, Waldbronn, Germany) with a variable wavelength UV detector, coupled to HP Chem Station utilizing the manufacturer's 5.01 software package.

All materials and methods used in chemistry part are also described in a formerly published work of our group [23, 25].

4.3 Procedures

4.3.1 Synthesis of target compounds

4.3.1.1 General procedure for pyrazoles synthesis

The experimental procedure developed by Stauffer et al. was applied for the preparation of target pyrazoles [24]. After completion of the reaction and work-up, the crude product was purified by flash chromatography or by passage through a short silica gel plug eluting with an ethyl acetate–hexane solvent system.

4.3.1.2 General demethylation procedure

The deprotection of the intermediate compounds was performed according to Stauffer et al. [24]. The crude phenolic products were obtained after purification by flash chromatography and/or recrystallization from MeOH–CH_2Cl_2 mixtures.

4.3.1.3 6-Methoxy-2-hydroxymethylen-3,4-dihydro-2H-naphthalen-1-one (1)

To a stirred solution of sodium hydride (27 mg, of 60 % dispersion in oil, 1.13 mmol) in 2 mL of dry DMF under an N_2 atmosphere, a solution of 6-methoxy-1-tetralone (0.20 g, 1.13 mmol) in 2 mL of dry THF was added dropwise. After being stirred for 4 h, ethyl formate (0.1 mL, 1.25 mmol) was added and the mixture was stirred for an additional 20 h. A saturated solution of NH_4Cl was added to quench the reaction mixture, and the product was extracted twice with ethyl acetate (2 × 15 mL). The joint organic layers were evaporated under vacuum, and the resulting orange solid was chromatographed (20 % EtOAc-hexanes) to give 0.18 g of the

desired product as amorphous yellow solid in 85 % yield. M.p. 95–96 °C (lit: 93 °C [26] and 97–100 °C [27]). – IR (KBr): 1688, 3211 cm^{-1}. – ^1H NMR (400 MHz, $CDCl_3$): δ = 2.52 (t, J = 6.6 Hz, 2H, 3-H), 2.85 (t, J = 6.6 Hz, 2H, 4-H), 3.83 (s, 3H, CH_3O), 6.71 (d, J = 2.2 Hz, 1H, 5-H), 6.84 (dd, J = 8.7, 2.2 Hz, 1H, 7-H), 7.93 (m, 2H, C=CH, 8-H), 14.70 (br s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 25.2, 28.4, 60.3, 114.2, 130.9, 166.7, 168.9 (–C=*C*–OH), 185.6 (C=O) ppm. – Anal. for $C_{12}H_{12}O_3$ (204.2): calcd. C 70.57, H 5.92; found: C 70.44, H 6.01.

4.3.1.4 7-Methoxy-2-(4-methoxyphenyl)-4,5-dihydro-2H-benzo[*g*]indazole (2) and 7-methoxy-1-(4-methoxyphenyl)-4,5-dihydro-1H-benzo[*g*]indazole (4)

Naphthalen-1-one **1** (0.3 g, 1.3 mmol) and 4-methoxyphenylhydrazine hydrochloride (0.9 g, 5.2 mmol) were reacted as outlined above to afford pyrazoles **2**, **4** as amorphous yellow solids (40 mg, 10 % and 0.32 g, 75 %, respectively) after passage through a short silica plug (20 % EtOAc-hexanes):

(**2**): M.p. 114–117 °C. – ^1H NMR (400 MHz, $CDCl_3$): δ = 2.82 (t, J = 6.8 Hz, 2H, 4-H), 2.96 (t, J = 6.8 Hz, 2H, 5-H), 3.81 (s, 3-H, CH_3O), 3.83 (s, 3H, CH_3O), 6.75 (d, J = 8.3 Hz, 1H, 9-H), 6.82 (d, J = 2.4 Hz, 1H, 6-H), 6.86 (dd, J = 8.3, 2.4 Hz, 1H, 8-H), 6.98 (d, J = 8.8 Hz, 2H, ArH), 7.62 (s, 1H, 3-H), 7.65 (d, J = 9.3 Hz, 2H, ArH) ppm. – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 24.8, 31.2, 55.9, 114.3, 120.4, 131.5, 136.8, 150.9, 155.8 ppm. – ^{15}N NMR (400 MHz, $CDCl_3$): δ = 285.2 (N-1), 212.2 (N-2) ppm. – Anal. for $C_{19}H_{18}N_2O_2$ (306.4): calcd. C 74.49, H 5.92, N 9.14; found: C, 74.35, H 5.76, N, 9.25.

(**4**): M.p. 105–106 °C. – ^1H NMR (400 MHz, $CDCl_3$): δ = 2.76 (t, J = 6.9 Hz, 2H, 4-H), 2.97 (t, J = 6.9 Hz, 2H, 5-H), 3.79 (s, 3H, CH_3O), 3.89 (s, 3H, CH_3O), 6.55 (dd, J = 8.6, 2.4 Hz, 1-H, 8-H), 6.76 (d, J = 8.6 Hz, 1H, 9-H), 6.87 (d, J = 2.4 Hz, 1H, 6-H), 6.99 (d, J = 8.8 Hz, 2H, ArH), 7. 44 (d, J = 8.8 Hz, 2H, ArH), 7.52 (s, 1H, 3-H) ppm. – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 24.5, 30.5, 55.6, 114.4, 122.8, 131.6, 136.7, 149.2, 159.5 ppm. – ^{15}N NMR (400 MHz, $CDCl_3$): δ, 205.4 (N-1), 305.3 (N-2) ppm. – Anal. for $C_{19}H_{18}N_2O_2$ (306.4): calcd. C 74.49, H 5.92, N 9.14; found: C, 74.31, H 5.76, N 9.37.

4.3.1.5 7-Methoxy-2-phenyl-4,5-dihydro-2H-benzo[*g*]indazole (3)

Naphthalen-1-one **1** (0.3 g, 1.29 mmol) and phenylhydrazine hydrochloride (0.93 g, 6.45 mmol) were reacted as outlined above to afford **3** as amorphous yellow solid (0.25 g, 74 %) after passage over a short silica plug (20 % EtOAc-hexanes). M.p. 110–113 °C. – ^1H NMR (400 MHz, $CDCl_3$): δ = 2.73 (t, J = 6.8 Hz, 2H, 4-H), 2.95 (t, J = 6.8 Hz, 2H, 5-H), 3.76 (s, 3H, CH_3O), 6.53 (dd, J = 8.7, 2.7 Hz, 1H, 8-H), 6.75 (d, J = 8.7 Hz, 1H, 9-H), 6.84 (d, J = 2.7 Hz, 1H, 6-H), 7.53 (s, 1H, 3-H), 7.41–7.49 (m, 5H, ArH) ppm. – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 24.9, 31.2, 55.5, 117.9, 125.2, 128.8, 131.4, 137.6, 152.3, 163.3 ppm. – ^{15}N NMR (400 MHz, $CDCl_3$) δ = 289.7 (N-1), 214.2 (N-2) ppm. – Anal. for $C_{18}H_{16}N_2O$ (276.3): calcd. C 78.24, H 5.84, N 10.14; found: C 78.51, H 5.74, N 10.35.

4.3.1.6 2-(4-Hydroxyphenyl)-4,5-dihydro-2H-benzo[*g*]indazol-7-ol (5)

A stirred CH_2Cl_2 solution of **2** (26 mg, 0.08 mmol) was deprotected using BBr_3, according to the general demethylation procedure. Purification by flash chromatography (50 % EtOAc-hexanes) afforded the title compound as yellow crystalline solid (18 mg, 82 %). M.p. 231–233 °C. – IR (KBr): ν = 3317 cm^{-1}. – ^1H NMR (400 MHz, [D_6]acetone): δ = 2.64 (t, J = 6.8 Hz, 2H, 5-H), 2.84 (t, J = 6.8 Hz, 2H, 4-H), 6.44 (dd, J = 8.5, 2.4 Hz, 1H, 8-H), 6.53 (d, J = 8.5 Hz, 1H, 9-H), 6.75 (d, J = 2.4 Hz, 1H, 6-H), 6.87 (d, J = 8.8 Hz, 2H, ArH), 7.19 (d, J = 8.8 Hz, 2H, ArH), 7.47 (s, 1H, H-3), 9.52 (br s, 1H, OH), 9.82 (br s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, [D_6]acetone): δ = 22.6, 27.5, 111.9, 115.7, 122.7, 128.5, 141.8, 152.6, 155.9 ppm. – Anal. for $C_{17}H_{14}N_2O_2$ (278.3): calcd. C 73.37, H 5.07, N 10.07; found: C 73.52, H 5.22, N 10.18.

4.3.1.7 2-Phenyl-4,5-dihydro-2H-benzo[*g*]indazol-7-ol (6)

A stirred CH_2Cl_2 solution of **3** (0.2 g, 0.72 mmol) was deprotected using BBr_3, according to the general demethylation procedure. Purification by flash chromatography (40 % EtOAc-hexanes) afforded the title compound as white crystalline solid (0.13 g, 87 %). M.p. 229–231 °C. – IR (KBr): ν = 3317 cm^{-1}. – ^1H NMR (400 MHz, [D_6]acetone): δ = 2.63 (t, J = 6.8 Hz, 2H, H-5), 2.84 (t, J = 6.8 Hz, 2H, H-4), 6.39 (dd, J = 8.7, 2.2 Hz, 1H, H-8), 6.49 (d, J = 8.7 Hz, 1H, H-9), 6.74 (d, J = 2.3 Hz, 1H, 6-H), 7.45–7.54 (m, 5H, ArH), 7.58 (s, 1H, 3-H), 9.53 (br s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, [D_6]acetone): δ = 24.5, 29.4, 111.9, 115.8, 122.4, 125.5, 128.6, 143.8, 154.3, 162.7 ppm. – Anal. for $C_{17}H_{14}N_2O$ (262.3): calcd. C 77.84, H 5.38, N 10.68; found: C 77.70, H 5.51, N 10.41.

4.3.1.8 4,5-Dihydro-1-(4-hydroxyphenyl)-1-benzo[*g*]indazol-7-ol (7)

A stirred CH_2Cl_2 solution of **4** (0.26 g, 0.85 mmol) was deprotected using BBr_3, according to the general demethylation procedure. Purification by flash chromatography (60 % EtOAc-hexane) afforded the title compound as white crystalline solid (0.19 g, 85 %). M.p. 259–261 °C. – IR (KBr):

ν = 3401 cm^{-1}. – ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 2.62 (t, J = 6.8 Hz, 2H, 5-H), 2.82 (t, J = 6.8 Hz, 2H, 4-H), 6.42 (dd, J = 8.5, 2.4 Hz, 1H, 8-H), 6.51 (d, J = 8.5 Hz, 1H, 9-H), 6.72 (d, J = 2.4 Hz, 1H, 6-H), 6.88 (d, J = 8.8 Hz, 2H, ArH), 7.20 (d, J = 8.8 Hz, 2H, ArH), 7.44 (s, 1H, 3-H), 9.53 (br s, 1H, OH), 9.83 (br s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, [D$_6$]acetone): δ = 24.8, 29.6, 111.6, 116.6, 123.8, 129.2, 143.8, 147.7, 159.7 ppm. – Anal. for $C_{17}H_{14}N_2O_2$ (278.3): calcd. C 73.37, H 5.07, N 10.07; found: C 73.50, H 5.16, N 9.91.

4.3.1.9 2-Hydroxymethylen-5-methoxy-indan-1-one (8)

Compound **8** was synthesized from 5-methoxy-1-indanone following the same process used for the preparation of compound **1**. After completion of the reaction and subsequent work-up, the resulting orange solid was chromatographed (ethyl acetate-hexane 4:1, *v*/*v*) to give 94 mg of the desired product as pale-yellow solid in 80 % yield. M.p. 135–136 °C (lit: 138–138.5 °C [28]). – IR (KBr): ν = 1692, 3301 cm^{-1}. – ^{1}H NMR (400 MHz, CDCl$_3$): δ = 3.45 (s, 2H, 3-H), 3.80 (s, 3H, CH$_3$O), 6.83–6.89 (m, 2H, 4-H, 6-H), 7.31 (s, 1H, C=CH), 7.68 (d, J = 8.3 Hz, 1H, 7-H), 9.82 (br s, 1H, OH). – ^{13}C NMR (400 MHz, CDCl$_3$): δ = 30.4, 58.4, 110.8, 127.1, 145.3, 165.2, 170.3 (–C=*C*–OH), 188.6 (C=O) ppm. – $C_{11}H_{10}O_3$ (190.2): calcd. C 69.46, H 5.30; found: C 69.34, H 5.19.

4.3.1.10 6-Methoxy-2-(4-methoxyphenyl)-2, 4-dihydro-2H-indeno[1,2-c]pyrazole (9)

Indan-1-one **8** (30 mg, 0.16 mmol) was reacted with 4-methoxyphenylhydrazine hydrochloride (0.14 g, 0.8 mmol) according to the general procedure to afford **9** as pale-yellow crystalline solid (41 mg, 85 %) after purification by flash chromatography (20 % EtOAc-hexanes). M.p. 95–96 °C. – ^{1}H NMR (400 MHz, CDCl$_3$): δ = 3.52 (s, 2H, 4-H), 3.80 (s, 3H, CH$_3$O), 3.81 (s, 3H, CH$_3$O), 6.77 (dd, J = 8.3, 2.2 Hz, 1H, 7-H), 7.03 (d, J = 8.7 Hz, 2H, ArH), 7.06 (d, J = 2.2 Hz, 1H, 5-H), 7.31 (d, J = 8.3 Hz, 1H, 8-H), 7.56 (s, 1H, 3-H), 7.58 (d, J = 8.7 Hz, 2H, ArH) ppm. – ^{13}C NMR (400 MHz, CDCl$_3$): δ = 24.5, 30.5, 58.9, 116.4, 122.5, 131.8, 136.6, 142.5, 149.2, 164.5 ppm. – ^{15}N NMR (CDCl$_3$): δ = 203.9 (N-1), 304.1 (N-2) ppm. – Anal. for $C_{19}H_{18}N_2O_2$ (292.3): calcd. C 73.95, H 5.52, N 9.58; found: C 74.01, H 5.59, N 9.45.

4.3.1.11 2-(4-Hydroxyphenyl)-2,4-dihydro-indeno[1,2-c] pyrazol-6-ol (10)

A stirred CH_2Cl_2 solution of **9** (40 mg, 0.14 mmol) was deprotected using BBr_3, according to the general demethylation procedure. Purification by flash chromatography (50 % EtOAc-hexane) afforded the title compound as yellow crystalline solid (32 mg, 90 %). M.p. 199–201 °C. – IR (KBr): ν = 3183 cm^{-1}. – ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 3.06 (s, 2H, H-4), 6.76 (dd, J = 8.5, 2.1 Hz, 1H, H-8), 7.03 (d, J = 8.5 Hz, 2H, ArH), 7.06 (d, J = 2.1 Hz, 1H, H-5), 7.28 (d, J = 8.5 Hz, 1H, H-8), 7.49 (s, 1H, H-3), 7.52 (d, J = 8.5 Hz, 2H, ArH), 8.58 (s, 1H, OH), 8.83 (s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, [D$_6$]acetone): δ = 35.9, 111.2, 116.4, 123.9, 129.2, 140.4, 147.5, 159.5 ppm. – Anal. for $C_{16}H_{12}N_2O_2$ (264.3): calcd. C 72.22, H 4.58, N 10.60; found: C 72.10, H 4.49, N 10.76.

4.3.1.12 6-Methoxy-2,2-di-(4-methoxybenzoyl)-3, 4-dihydro-2H-naphthalen-1-one (11)

6-Methoxy-1-tetralone (0.09 g, 0.51 mmol) in 5 mL THF was added dropwise to a stirred solution of 1 M LiHMDS (0.5 mL, 0.51 mmol) in THF over a period of 30 min. The resulting solution was stirred for a further 15 min prior to the addition of *p*-anisoyl chloride in THF. The reaction blend was stirred for 15 min and subsequently extracted using ethyl acetate (3 × 30 mL). The combined organic solvents were dried over $MgSO_4$ and concentrated in vacuo. Purification was achieved using silica gel chromatography. Elution with 10 % ethyl acetate in hexane yielded 80 mg (66 %) of the desired product as a pale-yellowish solid yield while 25 % of the unreacted tetralone was recovered. M.p. 133–134 °C. – IR (KBr): ν = 1702 cm^{-1}. – ^{1}H NMR (400 MHz, CDCl$_3$): δ = 2.69 (t, J = 6.6 Hz, 2H, 3-H), 2.88 (t, J = 6.6 Hz, 2H, 4-H), 3.60 (s, 3H, CH$_3$O), 3.80 (s, 3H, CH$_3$O), 3.82 (s, 3H, CH$_3$O), 6.53–6.71 (m, 6H, ArH), 7.01 (d, J = 8.3 Hz, 1H, ArH), 7.57–7.67 (m, 4H, ArH) ppm. – ^{13}C NMR (400 MHz, CDCl$_3$): δ = 26.2, 34.4, 62.1, 112.4, 116.5, 122.1, 125.8, 130, 162, 189, 198 ppm. – Anal. for $C_{27}H_{24}O_6$ (444.5): calcd. C 72.96, H 5.44; found C 72.77, H 5.69.

4.3.1.13 7-Methoxy-3-(4-methoxyphenyl)-2-phenyl-4, 5-dihydro-2H-benzo[*g*]indazole (12)

The triketone **11** (0.10 g, 0.22 mmol) was reacted with phenylhydrazine hydrochloride (0.13 g, 0.88 mmol) according to the general procedure above. Upon purification by flash chromatography (30 % EtOAc-hexanes) the title compound was obtained as amorphous orange solid (50 mg, 78 %). M.p. 161–163 °C. – ^{1}H NMR (400 MHz, CDCl$_3$): δ = 2.95 (m, 2H, 5-H), 3.01 (m, 2H, 4-H), 3.78 (s, 3H, CH$_3$O), 3.82 (s, 3H, CH$_3$O), 6.54 (dd, J = 8.6, 2.1 Hz, 1H, 8-H), 6.76 (d, J = 8.6 Hz, 1H, 9-H), 6.87 (d, J = 2.1 Hz, 1H, 6-H), 6.98 (d, J = 8.6 Hz, 2H, ArH), 7.43–7.52 (m, 3H, ArH), 7.57 (m, 2H, ArH), 7.70 (d, J = 8.6 Hz, 2H, ArH) ppm. – ^{13}C NMR (400 MHz, CDCl$_3$): δ = 23.7, 30.4, 57.5, 115.9, 122.2, 128.2, 129.3, 135.8, 138.5, 151.8, 160.7 ppm. – ^{15}N NMR (400 MHz, CDCl$_3$): δ = 203.6 (N-1), 299.7 (N-2) ppm. – $C_{25}H_{22}N_2O_2$ (382.5): calcd. C 78.51, H 5.80, N 7.32; found: C 78.22, H 5.64, N 7.42.

4.3.1.14 7-Methoxy-2,3-di-(4-methoxyphenyl) -4,5-dihydro-2H-benzo[*g*]indazole (13)

Triketone **11** (0.10 g, 0.22 mmol) and 4-methoxyphenylhydrazine hydrochloride (0.16 g, 0.37 mmol) were reacted as outline above to afford **13** as amorphous yellow solid after flash chromatographic purification (77 %). M.p. 141–143 °C. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.95–3.01 (m, 4H, 5-H, 4-H), 3.80 (s, 3H, CH_3O), 3.84 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 6.56 (dd, J = 8.5, 2.5 Hz, 1H, 8-H), 6.75 (d, J = 8.5 Hz, 1H, 9-H), 6.87 (d, J = 2.5 Hz, 1H, H-6), 6.98–7.02 (m, 4H, ArH), 7.57 (d, J = 8.7 Hz, 2H, ArH), 7.71 (d, J = 8.5 Hz, 2H, ArH) ppm. – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 23.4, 30.3, 57.1, 115.1, 122.5, 128.2, 129.3, 135.2, 138.9, 151.2, 157.8, 166.4 ppm. – ^{15}N NMR (400 MHz, $CDCl_3$): δ = 202.1 (N-1), 294.5 (N-2) ppm. – Anal. for $C_{26}H_{24}N_2O_3$ (412.5): calcd. C 75.71, H 5.86, N 6.79; found: C 75.84, H 5.73, N 6.63.

4.3.1.15 3-(4-Hydroxyphenyl)-1-phenyl-4,5-dihydro-2H-benzo[*g*]indazol-7-ol (14)

A stirred CH_2Cl_2 solution of **12** (23 mg, 0.06 mmol) was deprotected using BBr_3, according to the general demethylation procedure. Purification by flash chromatography (50 % EtOAc-hexanes) afforded the title compound as white crystalline solid (16 mg, 91 %). M.p. 165–166 °C. – IR (KBr): ν = 3201 cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.84–2.90 (m, 4H, 4-H, 5-H), 6.46 (dd, J = 8.6, 2.4 Hz, 1H, 8-H), 6.64 (d, J = 8.6 Hz, 1H, 9-H), 6.84 (d, J = 2.4 Hz, 1H, 6-H), 6.89 (d, J = 8.6 Hz, 2H, ArH), 7.41–7.46 (m, 1H, ArH), 7.48–7.53 (m, 3H, ArH), 7.59 (d, J = 9 Hz, 2H, ArH), 7.60–7.63 (m, 1H, ArH), 8.48 (bs, 1H, OH), 8.52 (bs, 1H, OH) ppm. –^{13}C NMR (400 MHz, $CDCl_3$): δ = 23.8, 30.5, 115.1, 122.1, 128.9, 129.6, 135.7, 138.9, 148.9, 150.2, 157.4 ppm. – Anal. for $C_{23}H_{18}N_2O_2$ (354.4): calcd. C 77.95, H 5.12, N 7.90; found: C 77.78, H 5.01, N 7.73.

4.3.1.16 2,3-Di-(4-hydroxyphenyl)-4,5-dihydro-2H-benzo[*g*]indazol-7-ol (15)

A stirred CH_2Cl_2 solution of **13** (23 mg, 0.06 mmol) was deprotected using BBr_3, according to the general demethylation procedure. Purification by flash chromatography (50 % EtOAc-hexanes) afforded the title compound as white crystalline solid (16 mg, 89 %). M.p. 301–303 °C. – IR (KBr): ν = 3470, 3501 cm^{-1}. – ^{1}H NMR (400 MHz, [D_6] acetone): δ = 2.86–2.96 (m, 4H, 4-H, 5-H), 6.45 (dd, J = 8.5, 2.4 Hz, 1H, 8-H), 6.70 (d, J = 8.5 Hz, 1H, 9-H), 6.85 (d, J = 2.4 Hz, 1H, 6-H), 6.92 (d, J = 8.9 Hz, 2H, ArH), 7.00 (d, J = 8.5 Hz, 2H, ArH), 7.32 (d, J = 8.9 Hz, 2H, ArH), 7.62 (d, J = 8.5 Hz, 2H, ArH), 8.50 (s, 1H, OH), 8.53 (s, 1H, OH), 8.86 (s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, [D_6]acetone): δ = 155, 152, 151, 138, 135, 129, 128, 122, 115, 28, 23 ppm. – Anal. for $C_{23}H_{18}N_2O_3$ (370.4): C 74.58, H 4.90, N 7.56; Found: C 74.35, H 5.04, N 7.67.

4.3.1.17 5-Methoxy-2,2-di-(4-methoxybenzoyl)-indan-1-one (16)

Compound **16** was synthesized from 5-methoxy-1-indanone following the same procedure used for the preparation of compound **11**. Purification was achieved using silica gel chromatography. Elution with 30 % ethyl acetate in hexane furnished 0.13 g of the desired product as a pale-yellowish solid in 62 % yield while 29 % of the unreacted indanone precursor was recovered. M.p. 121–122 °C. – IR (KBr): ν = 1742, 1737 cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.87 (s, 3H, CH_3O), 3.89 (s, 3H, CH_3O), 3.91 (s, 3H, CH_3O), 4.05 (s, 2H, 3-H), 6.64 (dd, J = 8.8, 2.1 Hz, 1H, 6-H), 6.85 (d, J = 2.1 Hz, 1H, 4-H), 6.91 (d, J = 9.0 Hz, 2H, ArH), 6.97 (d, J = 9.0 Hz, 2H, ArH), 7.00 (d, J = 9.0 Hz, 2H, ArH), 7.72 (d, J = 9.0 Hz, 2H, ArH), 8.23 (d, J = 8.8 Hz, 1H, H-7) ppm. – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 25.2, 62.6, 112.4, 116.2, 122.5, 125.7, 130.3, 155.5, 162.8, 192.1, 198.9 ppm. – Anal. for $C_{26}H_{22}O_6$ (430.5): calcd. C 72.55, H, 5.15; found: C 72.31, H 5.01.

4.3.1.18 6-Methoxy-3-(4-methoxyphenyl)-1-phenyl -1,4-dihydro-indeno[1,2-c]pyrazole (17)

Triketone **16** (50 mg, 0.12 mmol) and phenylhydrazine hydrochloride (0.16 g, 0.37 mmol) were reacted as outlined above to afford **17** as amorphous yellow solid after flash chromatographic purification (88 %). M.p. 149–150 °C. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.81 (s, 2H, 4-H), 3.83 (s, 3H, CH_3O), 3.88 (s, 3H, CH_3O), 6.83 (dd, J = 8.5, 2.3 Hz, 1H, 7-H), 6.99 (d, J = 8.7 Hz, 2H, ArH), 7.13 (d, J = 2.3 Hz, 1H, 5-H), 7.43 (d, J = 8.7 Hz, 2H, ArH), 7.54 (d, J = 8.7 Hz, 1H, 8-H), 7.56 (d, J = 8.8 Hz, 1H, ArH), 7.78 (d, J = 8.8 Hz, 2H, ArH), 7.90 (d, J = 8.8 Hz, 2H, ArH) ppm. – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 28.5, 57.8, 115.3, 122.6, 125.5, 129.9, 135.3, 138.8, 152.2, 163.5 ppm. – ^{15}N NMR (400 MHz, $CDCl_3$): δ = 202.8 (N-1), 301.7 (N-2) ppm. – Anal. for $C_{24}H_{20}N_2O_2$ (368.4): calcd. C 78.24, H 5.47, N 7.60; found: C 78.49, H 5.33, N 7.71.

4.3.1.19 6-Methoxy-1,3-bis(4-methoxyphenyl) -1,4-dihydro-indeno[1,2-c]pyrazole (18)

Triketone **16** (70 mg, 0.16 mmol) was reacted with 4-methoxyphenylhydrazine hydrochloride (0.12 g, 0.64 mmol) according to the general procedure to afford **18** as amorphous pale-yellow solid (30 mg, 90 %) after purification by flash chromatography (30 % EtOAc-hexanes). M.p. 157–159 °C. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.81 (s, 2H, 4-H), 3.83 (s, 3H, CH_3O), 3.85 (s, 3H, CH_3O), 3.89 (s, 3H, CH_3O),

6.79 (dd, J = 8.8, 2.4 Hz, 1H, 7-H), 6.98 (d, J = 8.4 Hz, 2H, ArH), 7.05 (d, J = 8.8 Hz, 2H, ArH), 7.12 (d, J = 2.4 Hz, 1H, 5-H), 7.35 (d, J = 8.8 Hz, 1H, 8-H), 7.65 (d, J = 8.8 Hz, 2H, ArH), 7.88 (d, J = 8.8 Hz, 2H, ArH). – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 26.8, 55.4, 115.7, 122.7, 124.2, 128.9, 135.3, 138.4, 152.6, 157.4, 163.5 ppm. – ^{15}N NMR ($CDCl_3$): δ = 201.9 (N-1), 302.4 (N-2) ppm. – Anal. for $C_{25}H_{22}N_2O_3$ (398.5): calcd. C 75.36, H 5.57, N 7.03; found: C 75.55, H 5.39, N 7.15.

4.3.1.20 1,4-Dihydro-3-(4-hydroxyphenyl)-1-phenylindeno[1,2-c]pyrazol-6-ol (19)

A stirred CH_2Cl_2 solution of **17** (35 mg, 0.09 mmol) was deprotected using BBr_3, according to the general demethylation procedure. Purification by flash chromatography (40 % EtOAc-hexanes) afforded 26 mg of the title compound as white crystalline solid in 82 % yield. M.p. 211–212 °C. – IR (KBr): ν = 3307 cm^{-1}. – ^{1}H NMR (400 MHz, [D_6]acetone): δ = 3.81 (s, 1H, 4-H), 6.81 (dd, J = 8.2, 2.3 Hz, 1H, 7-H), 6.95 (d, J = 8.5 Hz, 2H ArH), 7.14 (d, J = 2.3 Hz, 1H, 5-H), 7.42 (d, J = 8.5 Hz, 1H, ArH), 7.45 (d, J = 7.6 Hz, 1H, ArH), 7.62 (d, J = 8.2 Hz, 1H, 8-H), 7.65 (d, J = 7.6 Hz, 1H, ArH), 7.82 (d, J = 7.9 Hz, 2H, ArH), 7.85 (d, J = 8.5 Hz, 2H, ArH), 8.52 (s, 1H, OH), 8.60 (s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, [D_6]acetone): δ = 29.5, 115.4, 123.8, 128.4, 129.6, 135.3, 138.3, 148.8, 151.9, 157.1 ppm. – Anal. for $C_{22}H_{16}N_2O_2$ (340.4): C 77.63, H 4.74, N 8.23; found: C 77.75, H 4.86, N 8.39.

4.3.1.21 1,3-Bis-(4-Hydroxyphenyl)-1,4-dihydro-indeno[1,2-c]pyrazol-6-ol (20)

A stirred CH_2Cl_2 solution of **18** (19 mg, 0.05 mmol) was deprotected using BBr_3, according to the general demethylation procedure. Purification by flash chromatography (40 % EtOAc-hexanes) afforded the title compound as white crystalline solid (15 mg, 90 %). M.p. 279–281 °C. – IR: ν = 3245 cm^{-1}. – ^{1}H NMR (400 MHz, [D_6]acetone): δ = 3.81 (s, 1H, 4-H), 6.79 (dd, J = 8.2, 2.1 Hz, 1H, 7-H), 6.93 (d, J = 8.5 Hz, 2H, ArH), 7.07 (d, J = 8.8 Hz, 2H, ArH), 7.12 (d, J = 2.1 Hz, 1H, 5-H), 7.31 (d, J = 8.2 Hz, 1H, 8-H), 7.59 (d, J = 8.5 Hz, 2H, ArH), 7.80 (d, J = 8.5 Hz, 2H, ArH), 8.50 (s, 1H, OH), 8.53 (s, 1H, OH), 8.78 (s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, [D_6]acetone): δ = 29.9, 115.5, 118.4, 129.4, 135.7, 138.8, 148.9, 151.2, 158.5 ppm. – Anal. for $C_{22}H_{16}N_2O_3$ (356.4): calcd. C 74.15, H 4.53, N 7.86; found: C 74.38, H 4.68, N 7.97.

4.3.1.22 6-Benzyl-1,3-bis(4-methoxyphenyl)-1,4,5,6-tetrahydropyrrolo[2,3-*g*]indazole (22)

Compound **21** (0.5 g, 2.2 mmol) was dissolved in 7 mL of anhydrous toluene, cooled to 0 °C under argon and LiHMDS (2.3 mL, 1.0 M in THF, 2.3 mmol) and added via a syringe in one-pot under stirring. After approximately 1 min to allow the formation of the anion, *p*-anisoyl chloride (0.15 mL, 1.11 mmol) was added. The ice bath was removed, and after 1 min, 2 mL of AcOH was added. The reaction was stirred for an additional 2 min, and consecutively EtOH (10 mL), THF (5 mL), and an excess of 4-methoxyphenylhydrazine hydrochloride (1.73 g, 10 mmol) were added. The reaction mixture was refluxed for 15 min and completion of the reaction was verified by TLC. Then, the resulting solution was added to 5 mL of 1.0 M NaOH and extracted twice with EtOAc (2 × 20 mL). The combined organic fractions were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting residue was purified by flash column chromatography using a mixture of *n*-hexane–EtOAc (8:2) to provide compound **22** (0.61 g, 60 %) as pale-pink crystalline needles. M.p. 161 °C. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.79 (t, J = 8.0 Hz, 2H, 3-H), 3.04 (t, J = 8.3 Hz, 2H, 4-H), 3.85 (s, 3H, CH_3O), 3.89 (s, 3H, CH_3O), 5.08 (s, 2H, NCH_2Bz), 5.81 (d, J = 2.9 Hz, 1H, pyrrole), 6.51 (d, J = 2.9 Hz, 1H, pyrrole), 6.97 (d, J = 8.9 Hz, 2H, ArH), 7.01 (d, J = 8.9 Hz, 2H, ArH), 7.06 (d, J = 7.2 Hz, 2H, ArH), 7.26–7.33 (m, 3H, ArH), 7.58 (d, J = 8.9 Hz, 2H, ArH), 7.69 (d, J = 8.9 Hz, 2H, ArH) ppm. – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 22.8, 54.8, 95.4, 115.6, 119.2, 127.1, 132.5, 134.7, 146.9, 160.9 ppm. – Anal. for $C_{30}H_{27}N_3O_2$ (461.6): calcd. C, 78.07, H, 5.90, N, 9.10; found: C, 77.72, H, 5.72, N, 9.28.

4.3.1.23 4,4′-(6-Benzyl-4,5-dihydropyrrolo[2,3-*g*]indazole-1,3(6H)-diyl)diphenol (23)

Compound **23** was prepared from compound **22**, following the general demethylation protocol (85 % yield, pale-yellow crystals). M.p. 190 °C. – ^{1}H NMR (400 MHz, [D_6]acetone): δ = 2.80 (t, J = 8.0 Hz, 2H, H-3), 2.99 (t, J = 8.0 Hz, 2H, H-4), 5.21 (s, 2H, NCH_2Bz), 5.78 (d, J = 2.8 Hz, 1H, pyrrole), 6.68 (d, J = 2.6 Hz, 1H, pyrrole), 6.89 (d, J = 8.6 Hz, 2H, ArH), 6.99 (d, J = 8.5 Hz, 2H, ArH), 7.15 (d, J = 7.5 Hz, 2H, ArH), 7.26–7.34 (m, 3H, ArH), 7.44 (d, J = 8.6 Hz, 2H, ArH), 7.60 (d, J = 8.6 Hz, 2H, ArH), 8.40 (s, 1H, OH), 8.75 (s, 1H, OH) ppm. – ^{13}C NMR (400 MHz, [D_6]acetone): δ = 21.2, 54.5, 95.6, 115.1, 118.8, 127.4, 132.2, 134.3, 144.9, 155.5 ppm. – Anal. for $C_{28}H_{23}N_3O_2$ (433.5): calcd. C 77.58, H 5.35, N 9.69; found: C 78.34, H 5.22, N 10.18.

4.3.1.24 6-Benzyl-3-(3-chlorobenzo[b]thiophen-2-yl)-2,4,5,6-tetrahydropyrrolo[2,3-*g*]indazole (24)

Compound **24** was prepared following the same procedure presented for the synthesis of compound **22**. Initially

3-chlorobenzo[*b*]thiophene-2-carbonyl chloride was reacted with compound **21** 0.5 g (2.2 mmol), and subsequently condensed with hydrazine. The end product **24** (0.44 g, 49 % yield) was obtained in the form of yellow crystals. M.p. 144–146 °C. – ^{1}H NMR (400 MHz, [D_6] acetone): δ = 2.80 (t, J = 8.0 Hz, 2H, 3-H), 2.97 (t, J = 8.5 Hz, 2H, 4-H), 5.22 (s, 2H, $-CH_2$Bz), 6.38 (d, J = 2.6 Hz, 1H, 1-$H_{pyrrole}$), 6.82 (d, J = 2.8 Hz, 1H, 2-$H_{pyrrole}$), 7.15 (d, J = 7.5 Hz, 2H, ArH), 7.36 (m, 3H, ArH), 7.49 (t, J = 8.0 Hz, 1H, 5-H), 7.55 (t, J = 8.0 Hz, 1H, 6-H), 7.87 (d, J = 7.7 Hz, 1H, 4-H), 7.98 (d, J = 8.0 Hz, 1H, 3-H). – ^{13}C NMR (400 MHz, [D_6]acetone): δ = 18.9, 22.8, 54.9, 98.6, 116.8, 120.9, 125.1, 128.9, 134.1, 140.9 ppm. – Anal. for $C_{24}H_{18}ClN_3S$ (415.9): calcd. C 69.30, H 4.36, Cl 8.52, N 10.10, S 7.71; found: C 71.03, H, 4.16, Cl, 8.75, N 9.76, S 7.61.

4.4 Cell cultures and reagents

The human liver cancer HepG2 cell was donated by Dr. P. Liakos (University of Thessaly, Greece), and MCF-7 and cervix cancer HeLa cells were obtained from Dr. A.-M. Psarra (University of Thessaly, Greece). All cells were cultured in standard Dulbecco's modified Eagle's medium (DMEM, Gibko, UK), containing 10 % (*v*/*v*) fetal bovine serum, 2 mM L-glutamine (Gibko, UK), 100 units per mL of penicillin, and 100 units per mL of streptomycin (Gibko, UK) in plastic disposable tissue culture flasks at 37 °C in 5 % CO_2.

4.5 XTT assay for cell proliferation inhibition

Cell proliferation inhibition was assessed using the XTT assay kit (Roche, Germany). Briefly, cell lines were subcultured into a 96-well plate with 1×10^4 cells per well for HepG2 and MCF-7 cells and 5×10^3 cells per well for HeLa cells in the DMEM. After 24 h of incubation, the cells were treated with increasing concentrations (1–240 μM) of tested compounds in the serum-free DMEM for 24 h. A volume of 50 μL of XTT test solution, which was prepared by mixing 50 μL of XTT-labeling reagent with 1 μL of electron coupling reagent, was then added to each well. After 4 h of incubation, absorbance was measured at 450 nm and also at 690 nm as a reference wavelength in a BioTek ELx800 microplate reader (Winooski, VT, USA). The serum-free DMEM was used as a negative control. Also, the absorbance of each tested compound concentration alone in the serum-free DMEM and XTT test solution was tested at 450 nm. The absorbance values shown by the tested compounds alone were subtracted from those derived from cancer cell treatment with grape extracts. Data were calculated as a percentage of inhibition by the following formula:

$$\text{Inhibition}\,(\%) = [(\text{O.D.}_{\text{control}} - \text{O.D.}_{\text{sample}}) / \text{O.D.}_{\text{control}}] \times 100$$

where $\text{O.D.}_{\text{control}}$ and $\text{O.D.}_{\text{sample}}$ indicate the optical density of the negative control and the tested substances, respectively. The concentration of the tested compounds caused 50 % cellular proliferation inhibition of cancer cells (IC_{50}), which was calculated thereafter from the graph plotted percentage inhibition against extract concentration. All experiments were carried out in triplicate and at least on three separate occasions.

4.6 Assessment of VEGF expression levels

The human VEGF Quantakine ELISA kit (R&D Systems, MN, USA) was used for the quantitative determination of VEGF in culture supernates of HeLa cells. Cells were treated with vehicle control (1 % dimethyl sulfoxide [DMSO] in the cell culture medium) or different concentrations of tested compounds for 24 h. After treatment, cell culture supernatants were collected, and subjected to VEGF ELISA according to the manufacturer's protocol. Absorbance was measured using an automatic microplate reader BioTek ELx800 (Winooski, VT, USA) at 450 and 540 nm. The concentration of VEGF-A in each sample was calculated as in the published procedure by Myers et al. [29].

4.7 Tube formation assay

Briefly, 150 μL of an extracellular matrix solution (MatrigelTM, BD Biosciences, NJ, USA) was added to each well of a six-well plate and allowed to solidify for at least 30 min at 37 °C. Afterward, HMEC-1 were plated (1×10^5 cells per well) on the surface of the matrigel and treated with tested compounds (30 and 100 μM) or vehicle. Cells were incubated for 16 h, and then the effect of tested compounds on tubular morphogenesis was documented microscopically and photographed. Each experiment was repeated minimum three times.

4.8 Statistical analysis

All results are expressed as mean ± SD (n = 3). For statistical analysis, one-way ANOVA was applied followed by Dunnett's test for multiple pairwise comparisons. Spearman's correlation analysis examined dose response relationships. Differences were considered significant when

$p < 0.05$. All statistical analyses were accomplished with the SPSS software (version 14.0; SPSS).

5 Supplementary information

In the Supplementary Information, selected one- and two-dimensional NMR spectra are presented in order to facilitate the reading of this manuscript (http://dx.doi.org/10.1515/znb-2015-0053).

Acknowledgments: This work was supported by grants from Thalis project with acronym "SERMENCO" entitled "Development of Selective Estrogen Receptor Modulators as Agents against Menopause." This research project was co-funded by the Greek Operational Programme "Education and Lifelong Learning" and European Social Fund.

References

[1] American Cancer Society, *Cancer Facts and Figures 2014*, American Cancer Society, Atlanta, **2014**.
[2] A. Hollebecque, N. Houede, E. E. Cohen, C. Massard, A. Italiano, P. Westwood, W. Bumgardner, J. Miller, L. H. Brail, K. A. Benhadji, J. C. Soria, *Eur. J. Cancer* **2014**, *50*, 876.
[3] H. Geyer, K. Cannon, E. Knight, V. Fauble, J. Camoriano, R. Emanuel, R. Tibes, R. Mesa, *Leuk. Lymphoma* **2014**, *55*, 195.
[4] Q. Huang, T. W. Johnson, S. Bailey, A. Brooun, K. D. Bunker, B. J. Burke, M. R. Collins, A. S. Cook, J. J. Cui, K. N. Dack, J. G. Deal, Y. L. Deng, D. Dinh, L. D. Engstrom, M. He, J. Hoffman, R. L. Hoffman, P. S. Johnson, R. S. Kania, H. Lam, J. L. Lam, P. T. Le, Q. Li, L. Lingardo, W. Liu, M. W. Lu, M. McTigue, C. L. Palmer, P. F. Richardson, N. W. Sach, H. Shen, T. Smeal, G. L. Smith, A. E. Stewart, S. Timofeevski, K. Tsaparikos, H. Wang, H. Zhu, J. Zhu, H. Y. Zou, M. P. Edwards, *J. Med. Chem.* **2014**, *57*, 1170.
[5] C. Asche, M. Demeunynck, *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 247.
[6] A. Cimmino, A. Evidente, V. Mathieu, A. Andolfi, F. Lefranc, A. Kornienko, R. Kiss, *Nat. Prod. Rep.* **2012**, *29*, 487.
[7] J. C. O'Neill, H. E. Blackwell, *Comb. Chem. High Throughput Screening* **2007**, *10*, 857.
[8] I. M. Yonova, A. G. Johnson, C. A. Osborne, C. E. Moore, N. S. Morrissette, E. R. Jarvo, *Angew. Chem. Int. Ed.* **2014**, *53*, 2422.
[9] I. M. Yonova, C. A. Osborne, N. S. Morrissette, E. R. Jarvo, *J. Org. Chem.* **2014**, *79*, 1947.
[10] S. Grosse, V. Mathieu, C. Pillard, S. Massip, M. Marchivie, C. Jarry, P. Bernard, R. Kiss, G. Guillaumet, *Eur. J. Med. Chem.* **2014**, *84*, 718.
[11] S. Kumari, S. Paliwal, R. Chauhan, *Synth. Commun.* **2014**, *44*, 1521.
[12] E. Tzanetou, S. Liekens, K. M. Kasiotis, N. Fokialakis, S. A. Haroutounian, *Arch. Pharm. (Weinheim, Ger.)* **2012**, *345*, 804.
[13] M. S. Christodoulou, S. Liekens, K. M. Kasiotis, S. A. Haroutounian, *Bioorg. Med. Chem.* **2010**, *18*, 4338.
[14] M. S. Christodoulou, N. Fokialakis, S. Nam, R. Jove, A. L. Skaltsounis, S. A. Haroutounian, *Med. Chem.* **2012**, *8*, 779.
[15] H. Shih, L. Deng, C. J. Carrera, S. Adachi, H. B. Cottam, D. A. Carson, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 487.
[16] S. S. Moleele, J. P. Michael, C. B. de Koning, *Tetrahedron* **2008**, *64*, 10573.
[17] M. C. Kozlowski, B. J. Morgan, E. C. Linton, *Chem. Soc. Rev.* **2009**, *38*, 3193.
[18] F. C. E. Saraber, A. Baranovsky, B. J. M. Jansen, M. A. Posthumus, A. de Groot, *Tetrahedron* **2006**, *62*, 1726.
[19] M. Atobe, K. Naganuma, M. Kawanishi, A. Morimoto, K. Kasahara, S. Ohashi, H. Suzuki, T. Hayashi, S. Miyoshi, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6569.
[20] L. Huang, H. Miao, Y. Sun, F. Meng, X. Li, *Eur. J. Med. Chem.* **2014**, *87C*, 429.
[21] Cheminformatics, MOLINSPIRATION, http://www.molinspiration.com/cgi-bin/properties (accessed June 2015).
[22] M. J. Ahsan, J. Govindasamy, H. Khalilullah, G. Mohan, J. P. Stables, C. Pannecouque, E. De Clercq, *Bioorg. Med. Chem. Lett.* **2013**, *22*, 7029.
[23] K. M. Kasiotis, N. Fokialakis, S. A. Haroutounian, *Synthesis* **2006**, *11*, 1791.
[24] S. R. Stauffer, C. J. Coletta, R. Tedesco, G. Nishiguchi, K. Carlson, J. Sun, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *J. Med. Chem.* **2000**, *43*, 4934.
[25] F. Naoum, K. M. Kasiotis, P. Magiatis, S. A. Haroutounian, *Molecules* **2007**, *12*, 1259.
[26] B. Alcaide, *An. Quím. Ser. C* **1981**, *77*, 200.
[27] R. W. Hamilton, *J. Heterocycl. Chem.* **1976**, *13*, 545.
[28] W. S. Johnson, *J. Am. Chem. Soc.* **1946**, *66*, 218.
[29] T. Myers, S. Chengedza, S. Lightfoot, Y. Pan, D. Dedmond, L. Cole, Y. Tang, D. M. Benbrook, *Invest. New Drugs* **2009**, *27*, 304.

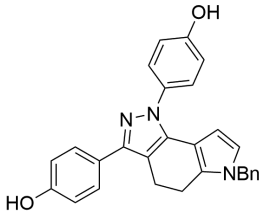
Supplemental Material: The online version of this article (DOI: 10.1515/znb-2015-0053) offers supplementary material, available to authorized users.

Graphical synopsis

Konstantinos M. Kasiotis,
Evangelia N. Tzanetou, Dimitrios
Stagos, Nikolas Fokialakis,
Eleni Koutsotheodorou, Dimitrios
Kouretas and Serkos A. Haroutounian

Novel conformationally constrained pyrazole derivatives as potential anti-cancer agents

DOI 10.1515/znb-2015-0053
Z. Naturforsch. 2015; x(x)b: xxx–xxx



Cell Viability IC_{50} (μM)		
HepG2	**HeLa**	**MCF-7**
3.0 ± 1.1	3.3 ± 0.9	5.0 ± 2.1