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Synthesis and biological activity of *N*,*N*-dialkylaminoalkyl-substituted bisindolyl and diphenyl pyrazolone derivatives

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Abstract—New compounds, structurally related to the potent protein kinase C inhibitor staurosporine, with a bisindolylpyrazolone framework and substituted on the pyrazolone nitrogens with *N*,*N*-dialkylaminoalkyl side chain, were synthesized and evaluated for growth-inhibitory properties in several human cell lines. Many showed inhibition of TNF-α production in response to the tumor promotor TPA on HL-60 cells. The apoptotic activity on HeLa cells has been examined for several of these compounds.

1. Introduction

Staurosporine, an indolo[2,3-*a*]carbazole which was discovered in the course of screening extracts of the bacterium *Streptomyces sp.*,[1] has become the 'lead' compound among protein kinase C (PKC) inhibitors. PKC is a suitable a target in anticancer drug design because its function is altered in some neoplasias, and this dysfunction has been related to uncontrolled proliferation.[2,3]

The staurosporine discovery stimulated a variety of medicinal chemistry programmes aimed at yielding staurosporine cogeners with higher inhibitory specificity for PKC.[4] One series of analogs was obtained by addition of substituents to the molecule (Fig. 1), e.g., addition of a hydroxyl group at the C_7 position of the lactam ring to furnish UCN-01,[5] and benzoylation of the amine in the glycoside ring to yield CGP 41251.[6] Both are currently progressing through clinical evalua-

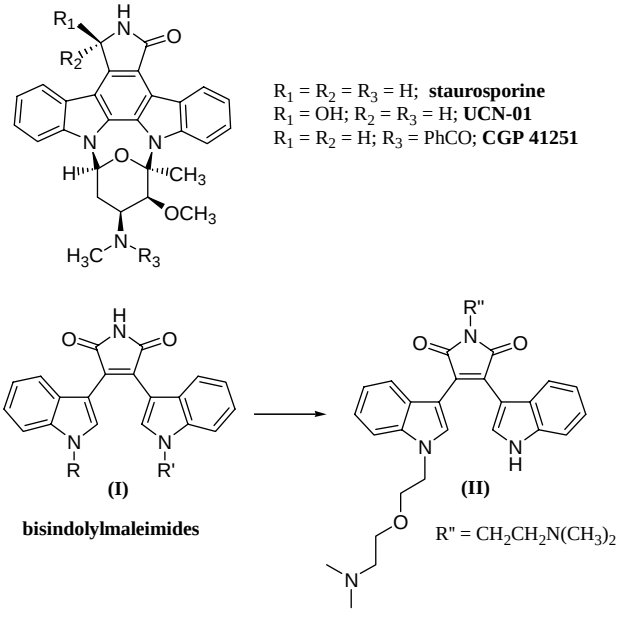

Figure 1. Staurosporine and related structures.

Keywords: Staurosporine; Bisindolopyrazolones; PKC; TNF-α; Apoptosis.
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tion as antitumoral agents.[7,8] Another successful modification furnished the bisindolylmaleimides. The starting point for the preparation of these potent and selective PKC inhibitors[9] came when it was decided to break the planarity of the aglycone structure of staurosporine[10,11] and when an extra carbonyl was added as a consequence of structure–activity relationships.[12] Consequently, various series of bisindolylmaleimide (**I**) compounds were designed. Many of them have appeared to be highly selective inhibitors of a broad spectrum of PKC.[13–15]

In a previous work,[16] we have synthesized a series of novel bisindolylmaleimide possessing a *N*,*N*-dialkylaminoalkyl side chain in their structure (**II**), and showed interesting activity and selectivity on human umbilical vein endothelial cells (HUVECs) proliferation, Figure 1.

In the course of structure–activity studies on PKC inhibitors as antitumoral agents and based on our previous findings, we have been engaged in new structural modification. We report here the synthesis of the compounds listed in Table 1, in which different side chains have been attached to a pyrazolone nucleous which permits to obtain three different regioisomers, we wished to determine if the additional nitrogen and the side chains contribute to the biological activity.

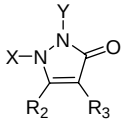
On the other hand, it has been demonstrated that when tumor necrosis factor-α (TNF-α) synthesis is stimulated by forbol addition, a signal cascade, where PKC is involved, occurs.[17] The most active compounds have been selected for the study of their regulatory activity on TNF-α production in human promelocytic leukemia (HL-60) cells. The apoptotic activity on human cervical carcinoma (HeLa) cells has also been studied to compare with staurosporine, a potent inducer of apoptosis.[18,19]

2. Results and discussion

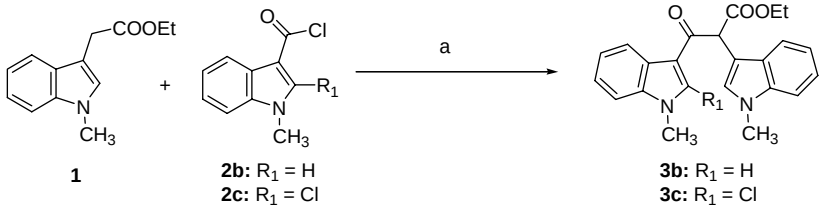
2.1. Chemistry

Compounds listed in Table 1 were obtained by the methods outlined in Schemes 1, 2. The synthesis of the pyrazolone-based compounds **4**–**6** required the preparation of the corresponding β-ketoesters **3** as starting material. Compound **3a** was prepared according to the procedure described in the literature.[20] The compounds **3b** and **3c** were prepared in the same procedure by reaction of ethyl-(1-methyl-1*H*-indol-3-yl)acetate **1** and the appropriate acyl chloride **2**. It was found that the yield was about 60% when the base was generated at −40 °C and when the reaction time was extended overnight.

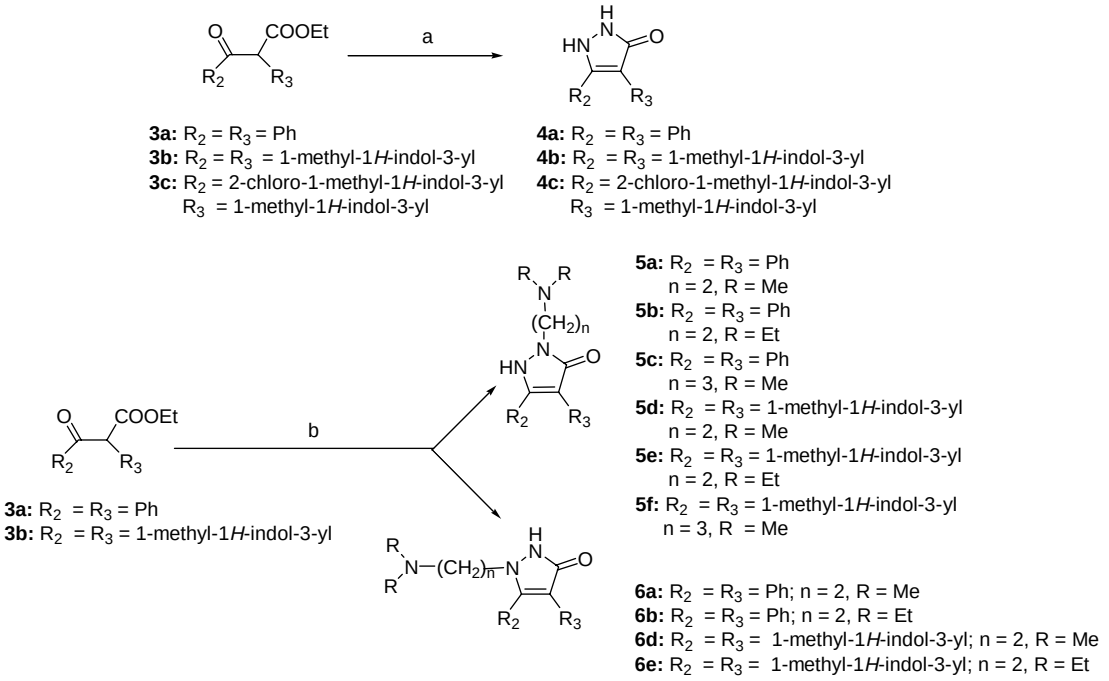
Table 1. Growth inhibitory properties for pyrazolone derivatives **4**, **5** and **6**



Compound	R_2	R_3	X	Y	IC_{50} (μM)		
					HT-29	HeLa	PC-3
4a	Ph	Ph	H	H	>50	>50	>50
4b	1-Me-1*H*-indol-3-yl	1-Me-1*H*-indol-3-yl	H	H	23.5	22.2	11.3
4c	2-Cl-1-Me-1*H*-indol-3-yl	1-Me-1*H*-indol-3-yl	H	H	>50	>50	>50
5a	Ph	Ph	H	$(CH_2)_2N(CH_3)_2$	>50	>50	>50
5b	Ph	Ph	H	$(CH_2)_2N(CH_2CH_3)_2$	>50	>50	>50
5c	Ph	Ph	H	$(CH_2)_3N(CH_3)_2$	>50	>50	>50
5e	1-Me-1*H*-indol-3-yl	1-Me-1*H*-indol-3-yl	H	$(CH_2)_2N(CH_2CH_3)_2$	14.4	12.8	46.7
6a	Ph	Ph	$(CH_2)_2N(CH_3)_2$	H	>50	>50	35.2
6b	Ph	Ph	$(CH_2)_2N(CH_2CH_3)_2$	H	27.5	>50	43.2
6d	1-Me-1*H*-indol-3-yl	1-Me-1*H*-indol-3-yl	$(CH_2)_2N(CH_3)_2$	H	21.0	17.4	35.2
6e	1-Me-1*H*-indol-3-yl	1-Me-1*H*-indol-3-yl	$(CH_2)_2N(CH_2CH_3)_2$	H	48.5	16.1	>50



Scheme 1. Synthesis of **3**. Reagents and condition: (a) BuLi/THF, diisopropylamine, −78 °C.



Scheme 2. Synthesis of **4**–**6**. Reagents and conditions: (a) Camphoric acid/EtOH, NH_2–NH_2, reflux, (b) camphoric acid/EtOH, H_2N-HN-$(CH_2)_n$ N-$(R)_2$, reflux.

In order to prepare **2b**, 1-methyl-1*H*-indole-3-carboxylic acid was refluxed with fresh distilled $SOCl_2$. However, the reaction led to the unexpected compound **2c**. The APCI-MS of corresponding acid of **2c** gave the characteristic isotope pattern from chloro, indicating its presence, and the substitution in the 2 position was confirmed by ^{1}H NMR spectrum. Therefore, we decided to carry out the reaction under milder conditions with $SOC1_2$ at room temperature and DMF to yield **2b**, which was used without further purification.

Thus, treatment of **3** with the appropriate hydrazine in ethanol at reflux in the presence of camphoric acid[14] yielded the corresponding pyrazolones **4**–**6**, Scheme 2. The *N*,*N*-dialkylaminoalkylhydrazines employed were obtained by reaction of the corresponding *N*,*N*-dialkylminoalkyl chlorides with excess of hydrazine monohydrate.[21] The condensation reaction could lead to regioisomers **5** and **6**. Both isomers could be separated by chromatography. Only when *N*,*N*-dimethylaminopropylhydrazine was used, **5c** and **5f** were obtained as unique compounds. The structure of the corresponding isomers was confirmed by NOE experiments, considering ^{1}H chemical shifts (positive NOE on signals of protons due to aromatic system upon irradiation of the CH_2 resonance with compounds **5a** and **6a**), as described in Figure 2.

But the unequivocal result was given by X-ray crystallographic analysis,[22] Figures 3 and 4. A complete characterization of the compounds **5d** and **5f** was performed but disappointingly these compounds darkened with time and they were not sufficiently stable for further experimental cytotoxic activity.

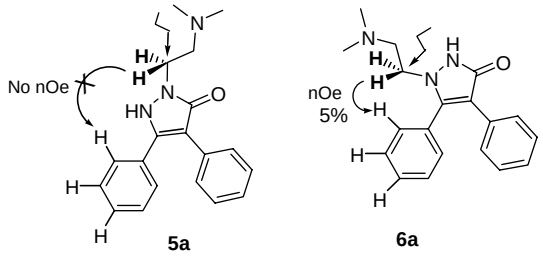


Figure 2. The NOE correlations used for the structural assignment of compounds **5a** and **6a**.

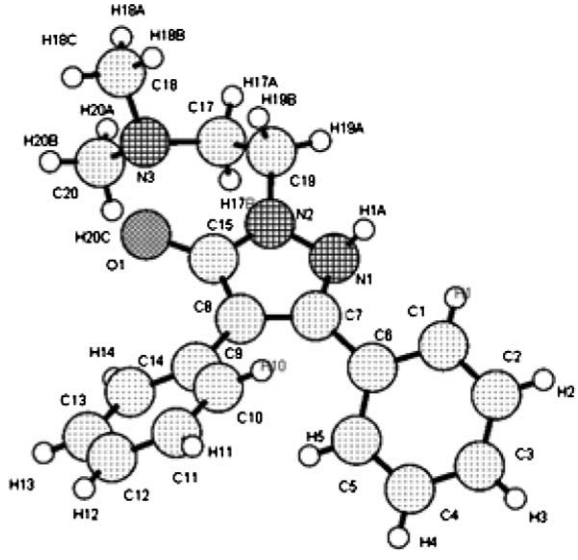


Figure 3. X-ray crystallographic analysis of **5a**.

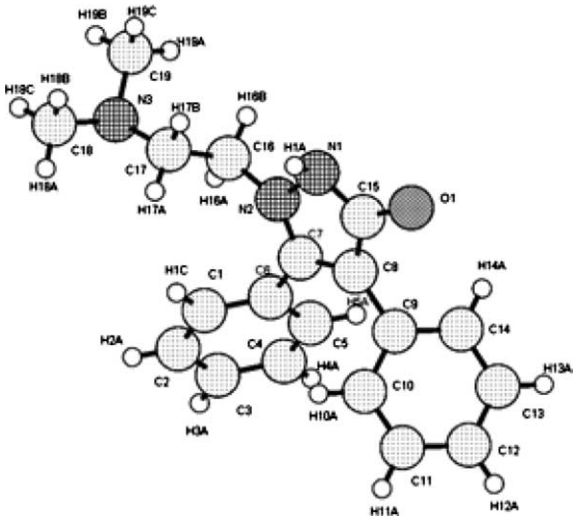
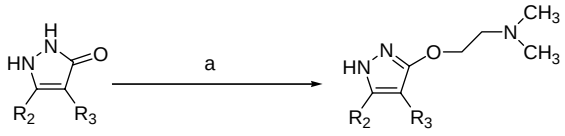


Figure 4. X-ray crystallographic analysis of **6a**.

An alternative, selective approach to N-substitution products was also possible via the reaction of **4a** and **4b** with *N*,*N*-dimethylaminoethanol under Mitsunobu conditions[23] shown in Scheme 3. This synthetic route did not permit access to the corresponding *N*,*N*-dialkylaminoalkylpyrazolone derivatives, so 3-alcoxy-pyrazol turned out to be the only product. The structure of all novel compounds was confirmed by mainly NMR spectroscopic methods and by X-ray crystallographic analysis, Figure 5.



4a: $R_2 = R_3 =$ Ph
4b: $R_2 = R_3 =$ 1-Me-1*H*-indol-3-yl

7a: $R_2 = R_3 =$ Ph
7b: $R_2 = R_3 =$ 1-Me-1*H*-indol-3-yl

Scheme 3. Synthesis of **7**. Reagents and conditions: (a) *N*,*N*-dimethylaminoethanol, Ph_3P, DIAD/THF, 0 °C.

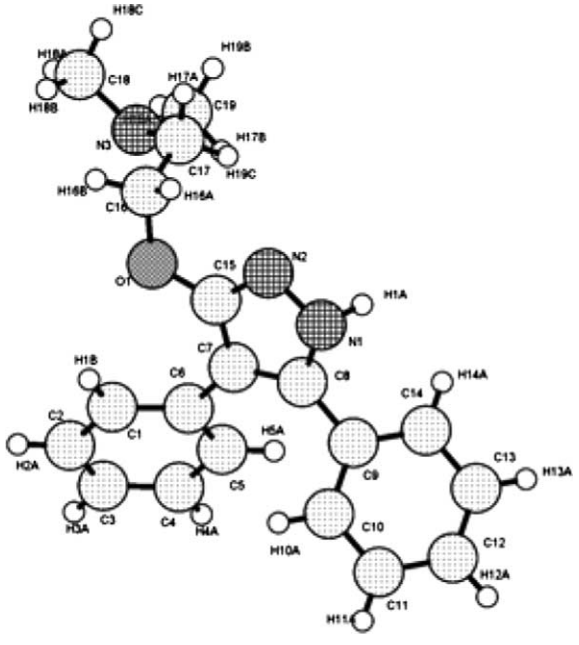


Figure 5. X-ray crystallographic analysis of **7a**.

3. Biological activity

In vitro cytotoxic potencies of the novel compounds **4**–**7** against human colon adenocarcinoma cell line (HT29), human cervical carcinoma (HeLa) and human prostate carcinoma (PC-3) are reported in Tables 1 and 2. Although none of them were as active as staurosporine, where IC_{50} values are in the nanomolar range as described in the literature,[24] these results indicate that some of the bisindole series have shown a moderate antiproliferative activity. Neither the introduction of side chains attached to the pyrazolone nitrogens nor the carbonyl group have significant effect on the activity. According to the IC_{50} observed, only **5e**, **6d**, **6e**, **7a** and **7b** were selected for proposed further biological evaluation.

The study of tumor promotion in rodent carcinogenesis using chemical tumor promoters has revealed various tumor promotion pathways, such as the 12-*o*-tetradecanoyl phorbol-13-acetate (TPA) pathway mediated through activation of protein kinase C.[25] It has also previously demonstrated that the treatment of human HL-60 promyelocytic leukemia cells with TPA is associated with induction of TNF-α.[26]

The study reported here has examined the effects on TNF-α promotion of those compounds, among which IC_{50} was promising in suggesting a link between the TPA pathway and endogenous tumor promotion pathway of TNF-α in HL-60 cells, which have been widely used as a model cell system for studying the biological roles of PKC.

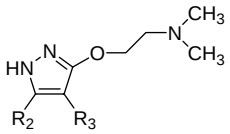
All selected compounds significantly reduce TNF-α production by TPA-stimulated HL-60 cells, Table 3. The amount of TNF-α produced in the presence of TPA alone was defined as 100%.[27]

In HeLa apoptosis assay,[28] two concentrations of each compound, a positive control (staurosporine, 20 μM) and a blank (phosphate saline buffer, pH 7.4), were used. Typical apoptotic bodies were observed 24 h after cells were treated with the selected compounds at 50 and 100 (μM). Compounds **5e**, **6e** and **7b** exhibit a considerable apoptotic capacity, Table 4.

4. Conclusions

In summary, different regioisomers, from diphenyl and bisindolyl, have been synthesized and completely characterized, possessing a dialkylaminoalkyl side chain in the pyrazolone nucleus. We explored the SAR for these new series of bisindolyl and diphenyl pyrazolone and the results obtained indicate that all the compounds possess

Table 2. Growth inhibitory properties for 3-alcoxy pyrazole derivatives **7**



Compound	R_2	R_3	IC_{50} (μM)		
			HT-29	HeLa	PC-3
7a	Ph	Ph	>50	21.8	23.8
7b	1-Me-1*H*-indol-3-yl	1-Me-1*H*-indol-3-yl	22.35	19.1	21.5

Table 3. TNF-α production

	TPA[a]
Compound	%
5e	34.90
6d	41.47
6e	18.98
7a	41.40
7b	28.12

[a] TPA, 12-*o*-tetradecanoylphorbol. The amount of TNF-α in the presence of TPA alone was defined as 100%.

Table 4. Apoptotic cells percentage

Compound	100 μM	50 μM
5e	54.7*	23.87*
6d	9.45	9.76
6e	45.6*	25.76*
7a	28.56*	11.15
7b	42.8*	36.6*
Control	8.65	
Staurosporine	63.2*	

Each result is the average of three recounts.
* Significantly different ($p < 0.05$).

a moderate antiproliferative activity in the micromolar range, the bisindole series were more potent than the diphenyl ones. These compounds could act as PKC inhibitors. Although PKC is involved in the assays studied (TNF-α production and apoptosis), it could not be confirmed that these compounds express their cytotoxicity via PKC inhibition. However, it should definitely be noted in future studies. The differential responses to individual inhibitors of PKC may be related to the multiple PKC isoforms and inhibitory and selectivity properties toward PKC isoforms are ongoing.

5. Experimental section

5.1. General methods

Thin-layer chromatography (TLC) was performed on E. Merck AL silicagel 60F254 plates and visualized under UV light. Flash column chromatography was conducted using E. Merck silica gel 60 (0.040–0.063 mm). Solvents employed were A (hexane/ethyl acetate, 1:1), B (ethyl acetate/methanol, 4:1), C (gradient of ethyl acetate/ethanol, 9:1 to ethyl acetate/ethanol/NH_3, 9:0.5:0.5). Infrared (IR) spectra were recorded with a Perkin-Elmer 1330 infrared spectrophotometer. All ^1H NMR and ^{13}C NMR spectra were recorded on a Brucker AM 300 MHz spectrometer in $CDC1_3$, with tetramethylsilane as internal standard. Chemical shifts are reported in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), quadruplet (c), quintuplet (q), multiplet (m). Elemental analyses were determined by the Microanalyses Service of the Complutense University of Madrid with an elemental Perkin Elmer 2400 CHN. Electrospray impact mass spectra (ESIMS) and atmosphere pressure chemical ionization mass spectra (APCI) were recorded on Brucker Daltonics ESQUIRE 3000. Melting points were obtained using a Stuart Scientific SMP3 apparatus and are uncorrected.

5.2. 1-Methyl-1*H*-indole-3-carbonyl chloride (2b)

To a solution of 1 mmol of 1-methyl-1*H*-indole-3-carboxylic acid with in 20 ml of fresh distilled $SOCl_2$, 3 drops of DMF were added, and the mixture was stirred for 20 h at room temperature. Then, the reaction mixture was evaporated in vacuo. The crude product was used without further purification.

5.3. 2-Chloro-1-methyl-1*H*-indole-3-carbonyl chloride (2c)

A mixture of 1 mmol of l-methyl-1*H*-indole-3-carboxylic acid in 20 ml of fresh distilled $SOCl_2$, was refluxed under stirring for 20 h. Then, the reaction mixture was evaporated in vacuo. The crude product was used without further purification.

5.4. General procedure for the preparation of β-ketoesters (3)

BuLi (2.2 mmol) was added to a solution of diisopropylamine (2.6 mmol) in THF (4 ml) under argon at −40 °C over 30 min with stirring. The solution was cooled at −78 °C and compound **1** (1 mmol) was added. After 1 h, the corresponding acyl halide 2 (1.2 mmol) was added dropwise, and stirred till room temperature.

5.5. Ethyl 2,3-bis-(1-methyl-1*H*-indol-3-yl)-3-oxopropanoate (3b)

3 g (13.81 mmol) of **1** and 3.28 g (17 mmol) of **2b** were used. Once the reaction was finished, the solvent was

evaporated in vacuo, and the crude product was dissolved in AcOEt, washed with 10% sodium bicarbonate solution, water and saturated sodium chloride solution, dried ($MgSO_4$) and evaporated. The product was purified by flash chromatography over silica gel using chromatography solvent A; yield 52%; mp 132–135 °C (from EtOH); IR (KBr) 1740, 1650 cm^{-1}; ^1H NMR δ: 1.27 (t, 3H, $J = 7.14$, CH_3), 3.76 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 4.24 (c, 2H, $J = 7.14$, CH_2), 5.70 (s, 1H, CH), 7.16–7.27 (m, 3H, ArH), 7.30–7.33 (m, 5H, ArH), 7.70 (d, 1H, $J = 7.14$, ArH), 7.82 (s, 1H, ArH); ^{13}C NMR δ: 14.10, 32.75, 33.42, 53.22, 61.43, 107.46, 109.40, 114.82, 118.32, 119.32, 121.59, 122.61, 122.71, 123.45, 123.67, 126.67, 127.08, 128.82, 136.08, 136.55, 137.23, 169.71, 187.97; Anal. $C_{23}H_{22}N_2O_3$ requires C, 73.78; H, 5.92; N, 7.48; found C, 73.70; H, 6.07; N, 7.16; APCIMS *m/z* 375.3 (M + H).

5.6. Ethyl 3-(2-chloro-1-methyl-1*H*-indol-3-yl)-2-(1-methyl-1*H*-indol-3-yl)-3-oxopropanoate (3c)

2.17 g (10 mmol) of **1** and 2.59 g (11.41 mmol) of **2c** were used. Once the reaction was completed, the solid obtained was filtered in vacuo, dissolved in $CHCl_3$, washed with 10% NH_4Cl, water and saturated sodium chloride solution, dried ($MgSO_4$) and evaporated. The crude product was washed with AcOEt and filtered in vacuo. Yield 32%; mp 167–169 °C; IR (KBr) 1750, 1650 cm^{-1}; ^1H NMR δ: 1.25 (t, 3H, $J = 7.14$, CH_3), 3.71 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 4.24 (c, 2H, $J = 7.14$, CH_2), 6.16 (s, 1H, CH), 7.13–7.22 (m, 3H, ArH), 7.25–7.37 (m, 4H, ArH), 7.68 (d, 1H, $J = 7.71$, ArH), 8.44 (d, 1H, $J = 5.55$, ArH); ^{13}C NMR δ: 14.10, 30.17, 32.70, 53.37, 61.32, 106.84, 109.28, 111.71, 118.81, 119.27, 121.57, 122.07, 123.07, 123.61, 126.16, 127.53, 128.74, 128.78, 131.56, 135.40, 136.70, 169.69, 188.40; Anal. $C_{23}H_{21}ClN_2O_3$ requires C, 67.56; H, 5.18; N, 6.85; found C, 67.27; H, 5.17, N, 6.99; APCIMS *m/z* 409.1 (M + H).

5.7. General procedure for the preparation of pyrazolones (4–6)

Camphoric acid (1 mmol) and the corresponding hydrazine (1 mmol) was added to a suspension of the appropriate β-ketoesther (**3**) (1 mmol) in EtOH (15 ml). After heating the mixture under reflux for 2 h, an excess of hydrazine (2 mmol) was added, and the solution stirred and heated under reflux for 24 h. The mixture was cooled, the solvent was evaporated in vacuo, and the crude product was dissolved in AcOEt, washed with 10% sodium bicarbonate solution, water and saturated sodium chloride solution, dried ($MgSO_4$), and evaporated. The products were purified by flash chromatography over silica gel.

5.8. 4,5-Bis-(1-methyl-1*H*-indol-3-yl)-1,2-dihydropyrazol-3-one (4b)

0.5 g of compound **3b** (1.33 mmol), 266 mg of hydrazine monohydrate (5.32 mmol) and 268 mg of acid camphoric (1.33 mmol) were used. Chromatography solvent A; yield 80%; mp 178–181 °C (from EtOH); IR (KBr) 3400, 3340 cm^{-1}; ^1H NMR δ: 3.61 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 6.97 (m, 2H, ArH), 7.13–7.23 (m, 3H, ArH), 7.26–7.32 (m, 3H, ArH), 7.38 (d, 1H, $J = 8.25$, ArH), 7.65 (d, 1H, $J = 7.68$, ArH); ^{13}C NMR δ: 32.47, 32.59, 96.67, 104.62, 105.00, 108.80, 109.30, 118.58, 119.88, 119.97, 120.67, 121.09, 121.90, 125.36, 127.36, 127.03, 128.34, 128.92, 136.45, 136.75, 162.12; Anal. $C_{21}H_{18}N_4O$ requires C, 73.67; H, 5.30; N, 16.36; found C, 73.55; H, 5.44; N, 16.58; ESIMS *m/z* 343.4 (M^+).

5.9. 5-(2-Chloro-1-methyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-1,2-dihydropyrazol-3-one (4c)

1.494 g of compound **3c** (3.65 mmol), 548 mg (10.95 mmol) of hydrazine monohydrate and 730 mg (3.65 mmol) of camphoric acid were used. In this case, the solid obtained was filtered in vacuo. Yield 59%; mp 272–275 °C (from DMF); IR (KBr) 3500, 3380 cm^1; ^1H NMR δ: 3.83 (s, 3H, CH_3), 4.24 (s, 3H, CH_3), 5.72 (s, 2H, 2NH), 6.97 (t, 1H, $J = 7.95$, ArH), 7.12–7.31 (m, 5H, ArH), 7.45 (d, 1H, $J = 7.95$, ArH), 7.52 (d, 1H, $J = 7.95$, ArH); 7.91 (d, 1H, $J = 7.32$, ArH); ^{13}C NMR δ: 29.96, 32.55, 100.01, 100.09, 102.26, 104.01, 109.42, 118.70, 119.12, 119.69, 121.21, 121.44, 122.82, 124.69, 126.47, 126.58, 128.22, 135.48, 136.51, 137.68, 155.27; Anal. $C_{24}H_{24}ClN_5O_2.C_3H_7NO$ requires C, 64.07; H, 5.38; N, 15.57; found C, 69.44; H, 5.36; N, 15.44; ESIMS *m/z* 377.1 (M^+).

5.10. 2-(2-Dimethylaminoethyl)-4,5-diphenyl-1,2-dihydropyrazol-3-one (5a)

1 g of compound **3a** (3.72 mmol), 1.15 g of *N*,*N*-dimethylaminoethylhydrazine (11.16 mmol) and 746 mg of acid camphoric (3.72 mmol) were used. Chromatography solvent B; yield 20%; mp 195–198 °C (from EtOH); IR (KBr) 3540, 3340 cm^{-1}; ^1H NMR δ: 2.60 (s, 6H, $2CH_3$), 3.06 (t, 2H, $J = 4.41$, CH_2), 4.30 (t, 2H, $J = 4.41$, CH_2), 7.13 (t, 1H, $J = 6.60$, ArH), 7.22–7.29 (m, 7H, ArH), 7.46 (m, 2H, ArH); ^{13}C NMR δ: 43.30, 45.76, 58.77, 100.43, 124.80, 127.28, 127.76, 127.86, 128.52, 133.27, 133.28, 133.83, 148.19, 155.28; Anal. $C_{19}H_{21}N_3O$ requires C, 74.24; H, 6.89; N, 13.67; found C, 73.98; H, 6.85; N, 13.51; ESIMS *m/z* 308.0 (M^+).

5.11. 1-(2-Dimethylaminoethyl)-4,5-diphenyl-2*H*-pyrazol-3-one (6a)

1 g of compound **3a** (3.72 mmol), 1.15 g of *N*,*N*-dimethylaminoethylhydrazine (11.16 mmol) and 746 mg of acid camphoric (3.72 mmol) were used. Chromatography solvent B; yield 10%; mp 178–180 °C (from acetone); IR (KBr) 3500, 3380 cm^{-1}; ^1H NMR δ: 2.17 (s, 6H, $2CH_3$), 2.73 (t, 2H, $J = 7.14$, CH_2), 3.97 (t, 2H, $J = 7.14$, CH_2), 7.09 (d, 1H, $J = 7.14$, ArH), 7.17 (t, 2H, $J = 7.68$, ArH), 7.28 (t, 2H, $J = 7.14$, ArH), 7.33 (m, 2H, ArH), 7.43 (m, 3H, ArH); ^{13}C NMR δ: 45.43, 46.30, 58.62, 66.80, 97.06, 104.72, 125.30, 127.93, 128.14, 128.95, 130.41, 132.05, 141.95, 159.44; Anal. $C_{19}H_{21}N_3O.H_2O$ requires C, 70.13; H, 7.12; N, 12.91; found C, 70.49; H, 6.73; N, 12.74; ESIMS *m/z* 308.0 (M^+).

5.12. 2-(2-Diethylaminoethyl)-4,5-diphenyl-1*H*-pyrazol-3-one (5b)

One gram of compound **3a** (3.72 mmol), 1.46 g of *N*,*N*-diethylaminoethylhydrazine (11.16 mmol) and 746 mg of acid camphoric (3.72 mmol) were used. Chromatography solvent B; yield 12%; mp 160–163 °C (from EtOH); IR (KBr) 3520, 3380 cm^{-1}; ^1H NMR δ: 1.25 (t, 6H, $J = 7.14$, $2CH_3$), 2.96 (c, 4H, $J = 7.14$, $2CH_2$), 3.14 (t, 2H, $J = 4.95$, CH_2), 4.33 (t, 2H, $J = 4.95$, CH_2), 7.11 (d, 1H, $J = 7.14$, ArH), 7.22–7.33 (m, 7H, ArH), 7.42-7.44 (m, 2H, ArH); ^{13}C NMR δ: 9.96, 46.49, 47.73, 53.83, 100.49, 124.95, 127.20, 128.00, 128.06, 128.80, 133.73, 134.67, 148.22, 153.97; Anal. $C_{21}H_{25}N_3O$ requires C, 75.19; H, 7.51; N, 12.53; found C, 74.85; H, 7.19; N, 12.66; ESIMS *m*/*z* 336.1 (M^+).

5.13. 1-(2-Diethylaminoethyl)-4,5-diphenyl-2*H*-pyrazol-3-one (6b)

One gram of compound **3a** (3.72 mmol), 1.46 g of *N*,*N*-diethylaminoethylhydrazine (11.16 mmol) and 746 mg of acid camphoric (3.72 mmol) were used. Chromatography solvent B; yield 4%; mp 157–159 °C (from acetone); IR (KBr) 3500, 3380 cm^{-1}; ^1H NMR δ: 0.95 (t, 6H, $J = 7.14$, $2CH_3$), 2.50 (c, 4H, $J = 7.14$, $2CH_2$), 2.82 (t, 2H, $J = 7.69$, CH_2), 3.97 (t, 2H, $J = 7.69$, CH_2), 7.09 (d, 1H, $J = 6{,}57$, ArH), 7.18 (t, 2H, $J = 7.69$, ArH), 7.25 (t, 2H, ArH), 7.33 (m, 2H, ArH), 7.44 (m, 3H, ArH); ^{13}C NMR δ: 11.75, 46.73, 47.66, 52.21, 125.33, 127.91, 128.11, 128.84, 128.99, 130.20, 132.00, 141.90, 159.39; Anal. $C_{21}H_{25}N_3O{\cdot}H_2O$ requires C, 71.36; H, 7.70; N, 11.89; found C, 71.09; H, 7.31; N, 11.56; ESIMS *m*/*z* 336.1 (M^+).

5.14. 2-(3-Dimethylaminopropyl)-4,5-diphenyl-1*H*-pyrazol-3-one (5c)

One gram of compound **3a** (3.72 mmol), 1.3 g of 3-(*N*,*N*-dimethylamino)propylhydrazine (11.16 mmol) and 746 mg of acid camphoric (3.72 mmol) were used. Chromatography solvent B; yield 16%; mp 174–176 °C (from EtOH); IR (KBr) 3520, 3400 cm^1; ^1H NMR δ: 2.10 (q, 2H, $J = 6.06$, CH_2), 2.36 (s, 6H, $2CH_3$), 2.51 (t, 2H, $J = 6.06$, CH_2), 4.18 (t, 2H, $J = 6.06$, CH_2), 7.12–7.17 (m, 1H, ArH), 7.23–7.32 (m, 5H, ArH), 7.35–7.38 (m, 2H, ArH), 7.48–7.52 (m, 2H, ArH); ^{13}C NMR δ: 26.26, 43.03, 43.39, 54.63,100.41, 124.79, 127.08, 127.89, 128.02, 128.08, 128.74, 133.92, 134.82, 148.14, 153.27; Anal. $C_{20}H_{23}N_3O$ requires C, 74.24; H, 7.21; N, 13.07; found C, 74.06; H, 7.18; N, 12.75; ESIMS *m*/*z* 322.1 (M^+).

5.15. 1-(2-Dimethylaminoethyl)-4,5-bis-(1-methyl-1*H*-indol-3-yl)-2*H*-pyrazol-3-one (6d)

One gram of compound **3b** (2.67 mmol), 826 mg of *N*,*N*-dimethylaminoethylhydrazine (8.01 mmol) and 535 mg of acid camphoric (2.67 mmol) were used. Chromatography solvent B; yield 9%; mp 258–261 °C (from EtOH); IR (KBr) 3500, 3360 cm^1; ^1H NMR δ: 2.13 (s, 6H, $2CH_3$), 2.71 (t, 2H, $J = 7.71$, CH_2), 3.66 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.98 (t, 2H, $J = 7.71$, CH_2), 6.87–6.91 (m, 2H, ArH), 7.03 (s, 1H, ArH), 7.11–7.13 (m, 2H, ArH), 7.20–7.37 (m, 4H, ArH), 7.44 (d, 1H,$J = 7.71$, ArH); ^{13}C NMR δ: 32.35, 32.64, 44.80, 46.26, 58.30, 100.23, 103.67, 105.22, 108.59, 109.37, 118.40, 119.45, 120.02, 120.61, 120.89, 121.94, 127.07, 127.16, 127.67, 129.47, 136.47, 136.50, 137.17, 140.98; Anal. $C_{25}H_{27}N_5O$ requires C, 72.61; H, 6.58; N, 16.94; found C, 72.74; H, 6.55; N, 16.73; ESIMS *m*/*z* 414.1(M^+).

5.16. 1-(2-Diethylaminoethyl)-4,5-bis-(1-methyl-1*H*-indol-3-yl)- 2*H*-pyrazol-3-one (6e)

One gram of compound **3c** (2.67 mmol), 1.05 g of *N*,*N*-diethylaminoethylhydrazine (8.01 mmol) and 535 mg of acid camphoric (2.67 mmol) were used. Chromatography solvent B; yield 9%; mp 195–197 °C (from EtOH); IR (KBr) 3520, 3360 cm^{-1}; ^1H NMR δ: 0.82 (t, 6H, $J = 7.14$, $2CH_3$), 2.38 (c, 4H, $J = 7.14$, $2CH_2$), 2.84 (t, 2H, $J = 7.71$, CH_2), 3.61 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 3.97 (t, 2H, $J = 7.71$, CH_2), 6.85–6.88 (m, 2H, ArH), 7.06–7.11 (m, 3H, ArH), 7.18 (d, 1H, $J = 8.25$, ArH), 7.23 (d, 1H, $J = 5.49$, ArH), 7.31 (d, 1H, $J = 8.25$, ArH), 7.45 (d, 1H, $J = 7.71$, ArH), 7.46 (d, 1H, $J = 8.25$, ArH); ^{13}C NMR δ: 11.72, 32.59, 32.62, 32.91, 47.15, 47.46, 52.48, 99.91, 104.51, 106.05, 108.59, 109.32, 118.49, 120.07, 120.13, 120.92, 121.47, 122.01, 127.33, 127.60, 129.51, 136.02, 136.65, 136.70, 160.08; Anal. $C_{27}H_{31}N_5O$ requires C, 73.44; H, 7.08; N, 15.86; found C, 73.76; H, 7.00; N, 15.80; ESIMS *m*/*z* 442.16 (M^+).

5.17. 2-(2-Diethylaminoethyl)-4,5-bis-(1-methyl-1*H*-indol-3-yl)-2*H*-pyrazol-3-one (5e)

One gram compound **3c** (2.67 mmol), 1.05 mg of *N*,*N*-diethylaminoethylhydrazine (8.01 mmol) and 535 mg of acid camphoric (2.67 mmol) were used. Chromatography solvent B; recrystallized from AcOEt/Hexane; yield 5%; IR (KBr) 3500, 3370 cm^{-1}; ^1H NMR δ: 1.15 (t, 6H, $J = 7.14$, $2CH_3$), 2.76 (c, 4H, $J = 7.14$, CH_2), 2.97 (dd, 2H, $J = 4.41$, $J = 4.92$, CH_2), 3.52 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 4.37 (dd, 2H, $J = 4.92$, $J = 4.41$, CH_2), 6.83 (s, 1H, ArH), 6.69 (t, 1H, $J = 7.14$, ArH), 7.06–7.25 (m, 5H, ArH), 7.30 (d, 1H, $J = 8.25$, ArH), 7.40 (d, 1H, $J = 7.68$, ArH), 8.20 (d, 1H,$J = 8.25$, ArH); ^{13}C NMR δ: 10.32, 11.95, 32.66, 32.79, 46.65, 47.30, 54.02, 107.23, 108.68, 108.87, 109.47, 119.40, 119.52, 121.40, 122.22, 126.55, 128.03, 128.13, 136.61, 137.04, 145.17; ESIMS *m*/*z* 442.16 (M^+).

5.18. General procedure for the preparation of 3-alcoxypyrazoles (7a–7c)

To a solution of 1 mmol of the corresponding pyrazolone (**4a**–**4b**), 1.5 mmol of Ph_3P, and 1.25 mmol of *N*,*N*-dimethylamino ethanol in 20 ml of THF, 1.5 mmol of diisopropylazodicarboxilate was added slowly, and the mixture was stirred for 20 h at room temperature. Then, 1 ml of MeOH was added, the mixture was poured onto 20 ml of water and was then exhaustively extracted with ether. The combined organic phases were washed with 2 M NaOH, water (several times), and

brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo.

5.19. *N*-[2-(4,5-Diphenyl-1*H*-pyrazol-3-yloxy)ethyl] dimethylamine (7a)

500 mg (2.11 mmol) of **4a** was used. Chromatography solvent C; yield 97%; mp 138–141 °C (from benzene/hexane); IR (KBr) 3500, 3350 cm^{-1}; ^1H NMR δ: 2.27 (s, 6H, $2CH_3$), 2.72 (t, 2H, $J = 6.06$, CH_2), 4.39 (t, 2H, $J = 6.06$, CH_2), 7.17 (d, 1H, $J = 6.06$, ArH), 7.24 (t, 2H, $J = 7.74$, ArH), 7.32 (m, 7H, ArH);^{13}C NMR δ: 45.70, 58.09, 67.00, 97.06, 104.19, 125.89, 127.05, 127.76, 128.03, 128.24, 128.45, 128.66, 129.09, 130.44, 131.64, 141.25, 161.06; Anal. $C_{19}H_{21}N_3O$ requires C, 74.24; H, 6.89; N, 13.67; found C, 74.17; H, 6.98; N, 13.63; APCIMS *m*/*z* 308.1 (M + H).

5.20. *N*-{2-[4,5-Bis-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrazol-3-yloxy]ethyl}dimethylamine (7b)

573 mg (1.67 mmol) of **4b** was used. Chromatography solvent C; yield 27%; mp 176–178 °C (from toluene); IR (KBr) 3520, 3440 cm^{-1}; ^1H NMR δ: 2.29 (s, 6H, $2CH_3$), 2.73 (t, 2H, $J = 6.06$, CH_2), 3.63 (s, 3H, CH_3), 3.75 (s, 3H, CH_3), 4.41 (t, 2H, $J = 6.06$, CH_2), 6.93-6.95 (m, 2H, ArH), 7.06 (s, 1H, ArH), 7.13–7.17 (t, 2H, ArH), 7.25.7.34 (m, 4H, ArH), 7.65 (d, 1H, J=7.14, ArH); ^{13}C NMR δ: 32.73, 32.82, 45.80, 58.19, 66.80, 97.06, 105.06, 105.32, 108.88, 109.55, 118.64, 119.80, 120.21, 121.31, 122.08, 125.24, 125.84, 127.07, 128.16, 128.36, 128.98, 136.61, 136.81, 161.81; Anal. $C_{25}H_{27}N_5O$ requires C, 72.61; H, 6.58; N, 16.94; found C, 72.52; H, 6.63; N, 16.58; APCIMS *m*/*z* 414.0 (M + H).

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