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Original article

Efficient microwave-assisted synthesis and antitumor activity of novel 4,4′-methylenebis[2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)phenols]

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ABSTRACT

Two new series of 4,4′-methylenebis[2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)phenols] **4** and methylenebis-2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-4,1-phenylene diacetates **5** have been efficiently prepared starting from the commercially available salicylaldehyde and where the key step corresponded to the microwave-assisted reaction of intermediates 3,3′-[methylenebis(6-hydroxy-3,1-phenylene)]bis(1-arylprop-2-en-1-ones) **3** with hydrazine hydrate or acetic acid/hydrazine hydrate respectively. Three unacetylated compounds type **4** presented interesting antitumor activities against a wide range of tumor cell lines.

1. Introduction

Pyrazoline derivates are five-member nitrogenated heterocyclic compounds associated with a broad spectrum of biological activities. Several pyrazoline derivatives have been investigated as inhibitors of mammalian monoamine oxidase (MAO)-A and-B [1] and antitumor agents. Baraldi et al. have synthesized and evaluated a series of hybrids of pyrazole analogues for *in vitro* cytotoxic activity against a variety of cancer cell lines [2], Antonini et al. reported the synthesis, antitumor cytotoxicity, and DNA-binding of novel *N*-5,2-di(ω-aminoalkyl)-2,6-dihydropyrazolo[3,4,5-*kl*]acridine-5-carboxamides using (ω-aminoalkyl)hydrazines as starting materials [3], Hernandez et al. recently were reported the synthesis of a novel pyrazolodibenzo[1,4]diazepines by a tandem sequence amine-exchange/heterocyclization starting from readily available enaminones and arylhydrazines, some of such derivatives shown a important antitumor activity [4]. Additionally, pyrazole derivatives have presented antibacterial activity [5] and pharmaceutical properties for using in the treatment of depression [6,7], hypotension [8] and inflammatory diseases [9]. Furthermore, compounds bearing two pyrazolinic rings have also shown significant pharmacological activities [10–12].

On the other hand, the development of high-throughput methodologies for drug discovery is highly demanded, and concretely the microwave-assisted reactions have gained considerable importance because of its simplicity, increasing reaction rates and cleaner product formation; including its well adaptation to a wide variety of experimental variants, such as solvent free approaches [13–21].

In this paper, we are reporting the synthesis of two series of 4,4′-methylenebis[2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)phenols] **4a–e** and methylenebis-2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-4,1-phenylene diacetates **5a–e** whose synthesis has been planned from the commercially available salicylaldehyde *via* the formation of key intermediates 3,3′-[methylenebis(6-hydroxy-3,1-phenylene)]bis(1-arylprop-2-en-1-ones) **3a–e**. The further microwave-assisted reaction with hydrazine hydrate, or with a mixture of hydrazine hydrate and acetic acid, respectively, afforded the desired products. Several of these compounds were tested for antitumor activity against 60 different human tumor cell lines, where products **4a**, **4c** and **4e** showed interesting values of GI_{50} and LC_{50}.

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2. Results and discussion

2.1. Chemistry

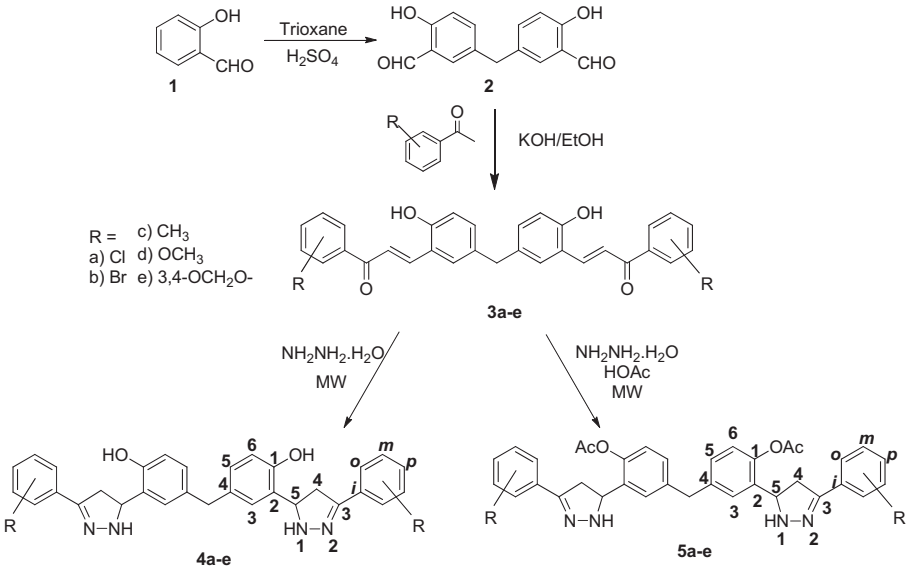
In order to obtain the key α,β-unsaturated intermediates as starting materials for the synthesis of the target products **4**, 5,5′-methylene-bis-salicylaldehyde **2** was obtained from the commercially available salicylaldehyde, which then underwent reaction with substituted acetophenones leading to the formation of the methylene-bis-chalcones **3a**–**e** [22,23]. Treatment of chalcones **3**, with hydrazine hydrate under microwave irradiation, at times no longer than 2 min, allowed the formation of the desired 4,4′-methylenebis[2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)phenols] **4a**–**e** in good to excellent yields. The addition of acetic acid to the above mixture under the same reaction conditions afforded the methylenebis-2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-4,1-phenylene diacetates **5a**–**e**, in just one step and in also good yields (Scheme 1).

The new series of compounds **4** and **5** were fully characterized by means of spectroscopic techniques such as FT-IR, 1D-, and 2D-NMR and EI-MS, as summarized in Experimental Section. The FT-IR spectra for compounds **4a**–**e** showed the characteristic absorption bands for OH and NH stretching at 3470–3451 cm^{-1} and 3418–3329 cm^{-1} respectively. As expected, compounds **5a**–**e** did not show the absorption band for the OH group, and did for the NH. The main feature to be remarked from the ^1H NMR spectra of compounds **4a**–**e** and **5a**–**e**, corresponded to typical signals of the pyrazoline ring. For example, both structures showed an AMX coupling system, involving the diastereotopic H-4 protons and the H-5 proton as showed in Experimental Section. All mass spectra of series **4** and **5** exhibited well-defined molecular ion peaks.

2.2. Anticancer activity

Three (**4a**, **4c** and **4e**) of the ten compounds proposed to NCI (National Cancer Institute of the United States) for screening were selected, and made the preliminary evaluation against the 60 tumor cell lines at a single dose of 1.0 μM. The output from the single dose screening was reported as a mean graph available for analysis by the COMPARE program. The results of the first assay showed that compounds **4a** and **4c** were active while compound **4e** was inactive.

Then, active compounds passed to a second stage in order to determine their cytostatic activity, they were evaluated at five concentration levels against various tumor cell lines like melanoma, leukemia and cancers of lung, colon, brain, breast, ovary, kidney or prostate. The test consisted of a 48 h continuous drug exposure protocol using sulforhodamide B (SRB) protein assay to estimate cell growth. Details of this evaluation method and the complementary information related with the activity pattern over all cell lines have been published [24–26]. Finally, the compound **4a** exhibited activity against 60 human tumor cell lines, with low values of $GI_{50} \sim 10^{-6}$ M in all cell lines, i.e. $GI_{50} = 9.48 \times 10^{-6}$ M for SK-MEL-28 (Melanoma), or $GI_{50} = 1.75 \times 10^{-6}$ M for K-562 (Leukemia), and in general interesting LC_{50} values, higher than 1×10^{-4} M. In a similar way, compound **4c** also showed an interesting activity against 32 human tumor cell lines, remarking that for SK-MEL-5 (Melanoma) with GI_{50}: 1.76×10^{-6} M and LC_{50}: 3.17×10^{-5} M. Consequently, compounds **4a** and **4c** exhibited significant activities, with GI_{50} ranges from 10^{-6} to 10^{-5} M. The cytotoxicities associated with the latter compounds, measured as LC_{50} are around 100 μM, for most cell lines, indicating a low toxicity of such compounds for normal human cell lines as required for potential antitumor agents (see Table 1).



R	Cl		Br		CH_3		OCH_3		3,4-OCH_3O-	
Comp.	**4a**	**5a**	**4b**	**5b**	**4c**	**5c**	**4d**	**5d**	**4e**	**5e**
Yield(%)	98	95	98	97	89	87	97	89	90	83
mp (°C)	260-262	164-166	265-267	194-196	267-269	145-147	250-252	156-158	279-281	179-180
t.r. (min)	1.5	1.0	1.5	1.0	1.5	1.0	2.0	1.5	2.0	1.5

Scheme 1. Synthesis of 4,4′-methylene-bis-2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)phenols **4a**–**f** and 4,4′-methylene-bis-2-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl-4,1-phenylene) diacetate **5a**–**f**.

Table 1
In vitro testing expressed as growth inhibition of cancer cell lines for compounds **4a** and **4c**.[a]

Panel/cell line	Compounds			
	4a		**4c**	
	GI_{50}[b] (μM)	LC_{50}[c] (μM)	GI_{50}[b] (μM)	LC_{50}[c] (μM)
Leukemia				
CCRF-CEM	1.94	>100	3.85	>100
HL-60(TB)	2.66	>100	–	–
K-562	1.75	>100	7.73	>100
MOLT-4	2.70	>100	–	–
RPMI-8226	5.09	>100	9.89	>100
Non-small cell lung				
SR	1.83	>100	–	–
A549/ATCC	2.45	>100	6.84	>100
EKVX	2.77	>100	–	–
HOP-62	4.16	>100	8.64	>100
HOP-92	3.39	>100	8.28	>100
NCI-H226	4.74	>100	8.28	>100
NCI-H23	3.30	>100	5.30	>100
NCI-H322M	4.17	>100	–	–
NCI-H460	2.15	>100	4.88	>100
NCI-H522	2.95	46.2	3.64	>100
Colon				
COLO 205	5.44	>100	–	–
HCC-2998	4.88	>100	–	–
HCT-116	1.85	>100	4.11	55.2
HCT-15	2.51	>100	3.64	>100
HT29	7.25	>100	–	–
KM12	3.09	>100	–	–
SW-620	3.29	>100	7.96	>100
CNS				
SF-268	3.22	>100	–	–
SF-295	1.47	>100	–	–
SF-539	5.82	>100	–	–
SNB-19	3.64	>100	6.89	>100
SNB-75	4.26	>100	4.98	>100
U251	1.80	>100	5.84	>100
Melanoma				
LOX IMVI	1.82	>100	7.38	>100
MALME-3M	5.08	>100	–	–
M14	4.45	>100	–	–
MDA-MB-435	3.21	>100	8.52	>100
SK-MEL-2	3.23	85.5	5.90	>100
SK-MEL-28	9.84	>100	–	–
SK-MEL-5	3.36	36.7	1.76	31.7
UACC-257	2.56	>100	–	–
UACC-62	3.16	43.6	–	–
Ovarian				
IGROV1	4.11	>100	–	–
OVCAR-3	2.59	>100	–	–
OVCAR-4	3.54	>100	5.36	>100
OVCAR-5	6.10	>100	–	–
OVCAR-8	2.46	>100	6.36	>100
NCI/ADR-RES	2.24	>100	7.39	>100
SK-OV-3	4.43	>100	–	–
Renal				
786-0	3.81	>100	8.39	>100
A498	5.37	>100	–	–
ACHN	3.63	>100	5.56	>100
CAKI-1	2.67	>100	–	–
RXF 393	3.99	>100	4.18	>100
SN12C	2.86	>100	8.84	>100
TK-10	5.34	>100	–	–
UO-31	3.04	>100	4.29	>100
Prostate				
PC-3	3.32	>100	–	–
DU-145	3.46	>100	–	–
Breast				
MCF7	3.04	>100	3.30	71.8
MDA-MB-231/ATCC	2.65	>100	–	–
HS 578T	2.76	>100	5.58	>100
BT-549	4.78	>100	4.66	>100
T-47D	3.79	>100	4.61	>100
MDA-MB-468	3.50	>100	2.97	>100

[a] Data obtained from NCI's *in vitro* disease-oriented human tumor cell lines screen [27].
[b] GI_{50} was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Determined at five concentration levels (100, 10, 1.0, 0.1 and 0.01 μM).
[c] LC_{50} is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells.

3. Conclusion

We have performed the synthesis of two novel series of bis-diarylpyrazoline derivates in a synthetic sequence starting from the commercially available salicylaldehyde, with a final key step which involved an efficient microwave-assisted cyclocondensation between methylene-bis-chalcones **3** and hydrazine in short reaction times and excellent yields. Biological assays on compounds **4a** and **4c** exhibited promising activities against different tumor cell lines with remarkable values of GI_{50}: 1.75–9.48 μM, along with their LC_{50} values (>100 μM). As compound **4a** showed an important activity against K-562 of Leukemia, it will be taken as a leader molecule for the development of new series of bispyrazoline derivates looking for improving the antitumor activity of this family of compounds.

4. Experimental

Commercially available starting materials, reagents and solvents were used as supplied. TLC analyses were performed on Merck silica gel 60 F_{254} aluminum plates. Melting points were determined in a Buchi Melting Point Apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were run on a Bruker AVANCE 400 spectrometer operating at 400 MHz and 100 MHz respectively, using dimethylsulfoxide-d_6 as solvent and tetramethylsilane as internal reference. The mass spectra were scanned on a Shimadzu GCMS-QP 2010 spectrometer (equipped with a direct inlet probe) and operating at 70 eV. The elemental analyses have been obtained using a Thermo Finnigan Flash EA1112 CHN (STIUJA) elemental analyzer. Microwave experiments were carried out on a focused microwave reactor (300 W CEM Discover™).

*4.1. General procedure for the synthesis of 4,4′-methylenebis[2-(3-aryl-4,5-dihydro-1H-pyrazol-5-yl)phenols] (**4a**–**e**)*

A mixture of 0.20 mmol of 3.3′-(5,5′-methylene-bis-2-hydroxyphenyl)-bis-1-arylprop-2-en-1-ones **3a**–**e** and 0.48 mmol of hydrazine hydrate was subjected to microwave irradiation in a closed vessel at 150 °C with a maximum power of 300 W for times no longer than two minutes. The reactions were followed by thin layer chromatography; the resulting mixture was cooled to room temperature and then water was added. The precipitate formed was filtered and washed several times with hot water and hexane.

*4.1.1. 4,4′-Methylenebis[2-(3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol] (**4a**)*

This compound was obtained as light brown solid, yield 98%, M.p. 260–262 °C, IR (KBr): $\upsilon = 3470$ for –OH, 3418 for –NH, 3020,

2980, 1650, 1587 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.65$ (dd, $J_2 = 1.3$ Hz, $J_{gem} = 15.8$ Hz, 2H), 3.36 (dd, $J_3 = 10.4$ Hz, $J_{gem} = 15.8$ Hz, 2H), 3.66 (s, 2H), 4.94 (dd, $J_2 = 1.3$ Hz, $J_3 = 10.4$, 2H), 6.67–7.59 (m, 14H), 7.11–7.18 (bs, 2H, OH), 9.34 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 39.5$, 40.2, 59.3, 115.4, 127.4, 128.4, 128.5, 128.5, 128.9, 132.4, 132.7, 132.8, 148.6, 153.3 ppm; EI-MS (*m*/*z*, %): 560 [M + 4] (2), 558 [M + 2] (13), 556 [M^+] (20), 376 (13), 198 (100), 178 (67). Anal. Calcd. for $C_{31}H_{26}Cl_2N_4O_2$: C, 66.79; H, 4.70; N, 19.49. Found: C, 66.69; H, 4.77; N, 19.43.

4.1.2. 4,4′-Methylenebis[2-(3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol] (**4b**)

This compound was obtained as light brown solid, yield 98%, M.p. 265–267 °C, IR (KBr): $\upsilon = 3451$ for –OH, 3329 for –NH, 3017, 2980, 1657, 1586 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.65$ (dd, $J_2 = 1.1$ Hz, $J_{gem} = 15.6$ Hz, 2H), 3.38 (dd, $J_3 = 10.4$ Hz, $J_{gem} = 15.6$ Hz, 2H), 3.66 (s, 2H), 4.91 (dd, $J_2 = 1.1$ Hz, $J_3 = 10.4$ Hz, 2H), 6.68–7.52 (m, 14H), 7.10–7.17 (bs, 2H, OH), 9.36 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 39.4$, 40.4, 59.3, 115.4, 121.4, 127.3, 128.4, 128.5, 131.5, 131.8, 132.4, 133.0, 148.6, 153.3 ppm; EI-MS (*m*/*z*, %): 648 [M + 4] (6), 646 [M + 2] (10), 644 [M^+] (5), 420 (19), 222 (100), 198 (53). Anal. Calcd. for $C_{31}H_{26}Br_2N_4O_2$: C, 57.60; H, 4.05; N, 8.67. Found: C, 57.67; H, 4.12; N, 8.61.

4.1.3. 4,4′-Methylenebis[2-(3-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol] (**4c**)

This compound was obtained as light brown solid, yield 89%, M.p. 267–269 °C, IR (KBr): $\upsilon = 3456$ for –OH, 3330 for –NH, 3024, 2979, 1658, 1510 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.28$ (s, 6H), 2.65 (dd, $J_2 = 2.0$ Hz, $J_{gem} = 16.2$ Hz, 2H), 3.36 (dd, $J_3 = 10.8$ Hz, $J_{gem} = 16.2$ Hz, 2H), 3.67 (s, 2H), 4.91 (dd, $J_2 = 2.0$ Hz, $J_3 = 10.8$ Hz, 2H), 6.67–7.49 (m, 14H), 7.19–7.25 (bs, 2H, OH), 9.36 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 21.3$, 39.8, 39.8, 59.3, 115.5, 125.9, 127.5, 128.3, 128.5, 129.5, 131.0, 132.4, 138.0, 150.4, 153.4 ppm; EI-MS (*m*/*z*, %): 516 [M^+] (22), 356 (12), 198 (53), 158 (100). Anal. Calcd. for $C_{34}H_{32}N_4O_2$: C, 76.72; H, 6.24; N, 10.84. Found: C, 76.66; H, 6.19; N, 10.76.

4.1.4. 4,4′-Methylenebis[2-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol] (**4d**)

This compound was obtained as light brown solid, yield 97%, M.p. 250–252 °C, IR (KBr): $\upsilon = 3463$ for –OH, 3352 for –NH, 3008, 2978, 1656, 1550 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.64$ (dd, $J_2 = 1.1$ Hz, $J_{gem} = 16.4$ Hz, 2H), 3.36 (dd, $J_3 = 11.0$ Hz, $J_{gem} = 16.4$ Hz, 2H), 3.68 (s, 2H), 3.75 (s, 6H), 4.89 (dd, $J_2 = 1.1$ Hz, $J_3 = 11.0$ Hz, 2H), 6.67–7.54 (m, 14H), 7.11–7.18 (bs, 2H, OH), 9.38 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 40.0$, 40.3, 55.6, 59.3, 114.4, 115.5, 126.4, 127.4, 127.5, 128.3, 128.4, 128.5, 132.4, 153.4, 159.9 ppm; EI-MS (*m*/*z*, %): 548 [M^+] (32), 372 (100), 198 (49), 174 (60). Anal. Calcd. for $C_{33}H_{32}N_4O_4$: C, 72.24; H, 5.88; N, 10.21. Found: C, 72.30; H, 5.94; N, 10.17.

4.1.5. 4,4′-Methylenebis[2-(3-(1,3-benzodioxol-5-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol] (**4e**)

This compound was obtained as light brown solid, yield 90%, M.p. 279–281 °C, IR (KBr): $\upsilon = 3470$ for –OH, 3359 for –NH, 3006, 2978, 1667, 1550 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.65$ (dd, $J_2 = 0.9$ Hz, $J_{gem} = 16.3$ Hz, 2H), 3.36 (dd, $J_3 = 10.8$ Hz, $J_{gem} = 16.3$ Hz, 2H), 3.67 (s, 2H), 4.93 (dd, $J_2 = 0.9$ Hz, $J_3 = 10.8$ Hz, 2H), 6.06 (s, 4H), 6.67–7.59 (m, 14H), 7.12–7.19 (bs, 2H, OH), 9.39 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 39.5$, 40.3, 59.3, 101.2, 115.4, 115.8, 117.3, 127.4, 127.6, 127.9, 128.5, 128.9, 129.9, 153.3, 160.0 ppm; EI-MS (*m*/*z*, %): 576 [M^+] (23), 198 (47), 188 (100). Anal. Calcd. for $C_{33}H_{28}N_4O_6$: C, 68.74; H, 4.89; N, 9.72. Found: C, 68.67; H, 4.82; N, 9.68.

4.2. General procedure for the synthesis of methylenebis-2-(3-aryl-4,5-dihydro-1H-pyrazol-5-yl)-4,1-phenylene diacetates **5a**–**e**

A mixture of 0.20 mmol of 3.3′-(5,5′-methylene-bis-2-hydroxyphenyl)-bis-1-arylprop-2-en-1-ones **3a**–**e**, 0.48 mmol of hydrazine hydrate and 0.40 mmol of acetic acid was subjected to microwave irradiation in a closed vessel at 180 °C with a maximum power of 300 W for times no longer than 2 min. The reactions were followed by thin layer chromatography; the resulting mixture was cooled to room temperature and then water was added. The precipitate formed was filtered and washed twice with hot water and hexane.

4.2.1. Methylenebis-2-(3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-4,1-phenylene diacetates (**5a**)

This compound was obtained as light brown solid, yield 95%, M.p. 164–166 °C, IR (KBr): $\upsilon = 3387$ for –NH, 3015, 2983, 1696 for C=O, 1670, 1523 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.23$ (s, 6H), 2.95 (dd, $J_2 = 1.5$ Hz, $J_{gem} = 14.3$ Hz, 2H), 3.60 (s, 2H), 3.70 (dd, $J_3 = 10.3$ Hz, $J_{gem} = 14.3$ Hz, 2H), 5.53 (dd, $J_2 = 1.5$ Hz, $J_3 = 10.3$ Hz, 2H), 6.62–7.74 (m, 14H), 9.40 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 22.1$, 40.0, 40.5, 41.2, 115.8, 126.6, 126.9, 127.8, 128.5, 128.7, 129.2, 130.1, 135.1, 152.5, 154.0, 167.8 ppm; EI-MS (*m*/*z*, %): 644 [M + 4](3), 642 [M + 2](12), 640 [M^+] (16), 580 (31), 280 (100). Anal. Calcd. for $C_{35}H_{30}Cl_2N_4O_4$: C, 65.53; H, 4.71; N, 8.73. Found: C, 65.59; H, 4.72; N, 8.66.

4.2.2. Methylenebis-2-(3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-4,1-phenylene diacetates (**5b**)

This compound was obtained as light brown solid, yield 97%, M.p. 194–196 °C, IR (KBr): $\upsilon = 3380$ for –NH, 3025, 2982, 1696 for C=O, 1674, 1528 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.23$ (s, 6H), 2.95 (dd, $J_2 = 1.0$ Hz, $J_{gem} = 16.3$ Hz, 2H), 3.60 (s, 2H), 3.71 (m, $J_3 = 11.5$ Hz, $J_{gem} = 16.3$ Hz, 2H), 5.53 (dd, $J_2 = 1.0$ Hz, $J_3 = 11.5$ Hz, 2H), 6.62–7.65 (m, 14H), 9.41 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 22.1$, 40.2, 40.4, 41.2, 115.8, 126.6, 126.8, 127.7, 128.5, 128.9, 129.0, 131.1, 135.0, 152.5, 154.1, 167.8 ppm; EI-MS (*m*/*z*, %): 732 [M + 4] (26), 730 [M + 2] (47), 728 [M^+] (24), 668 (100), 280 (71). Anal. Calcd. for $C_{35}H_{30}Br_2N_4O_4$: C, 57.55; H, 4.14; N, 7.67. Found: C, 57.61; H, 4.12; N, 7.60.

4.2.3. Methylenebis-2-(3-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-4,1-phenylene diacetates (**5c**)

This compound was obtained as light brown solid, yield 87%, M.p. 145–147 °C, IR (KBr): $\upsilon = 3384$ for –NH, 3002, 2989, 1699 for C=O, 1664, 1539 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.23$ (s, 6H), 2.31 (s, 6H), 2.93 (dd, $J_2 = 1.1$ Hz, $J_{gem} = 15.8$ Hz, 2H), 3.59 (s, 2H), 3.68 (dd, $J_3 = 10.7$ Hz, $J_{gem} = 15.8$ Hz, 2H), 5.50 (dd, $J_2 = 1.1$ Hz, $J_3 = 10.7$ Hz, 2H), 6.61–7.62 (m, 14H), 9.39 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 22.0$, 22.1, 40.0, 40.3, 41.4, 115.5, 126.5, 126.9, 127.9, 128.0, 128.4, 129.7, 132.1, 140.3, 152.5, 154.9, 167.6 ppm; EI-MS (*m*/*z*, %): 600 [M^+] (41), 540 (100), 280 (31). Anal. Calcd. for $C_{37}H_{36}N_4O_4$: C, 73.98; H, 6.04; N, 9.33. Found: C, 73.91; H, 6.12; N, 9.39.

4.2.4. Methylenebis-2-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-4,1-phenylene diacetates (**5d**)

This compound was obtained as light brown solid, yield 89%, M.p. 156–158 °C, IR (KBr): $\upsilon = 3312$ for –NH, 3009, 2980, 1698 for C=O, 1663, 1514 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.22$ (s, 6H), 2.92 (dd, $J_2 = 1.2$ Hz, $J_{gem} = 16.8$ Hz, 2H), 3.59 (s, 2H), 3.77 (dd, $J_3 = 11.0$ Hz, $J_{gem} = 16.8$ Hz, 2H), 3.85 (s, 6H), 5.51 (dd, $J_2 = 1.2$ Hz, $J_3 = 11.0$ Hz, 2H), 6.60–7.79 (m, 14H), 9.39 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 22.0$, 40.0, 40.2, 41.5, 46.4, 114.6, 115.8, 121.9, 126.3, 128.4, 128.6, 137.1, 138.1, 145.7, 151.7, 162.9, 169.0 ppm; EI-MS (*m*/*z*,

%): 632 [M^+] (5), 572 (8), 280 (100). Anal. Calcd. for $C_{37}H_{36}N_4O_6$: C, 70.24; H, 5.74; N, 8.86. Found: C, 70.18; H, 5.82; N, 8.79.

*4.2.5. Methylenebis-2-[3-(1,3-benzodioxol-5-yl)-4,5-dihydro-1H-pyrazol-5-yl]-4,1-phenylene diacetate (**5e**)*

This compound was obtained as light brown solid, yield 83%, M.p. 179–180 °C, IR (KBr): $\upsilon = 3310$ for −NH, 3000, 2967, 1694 for C═O, 1662, 1573 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.25$ (s, 6H), 2.95 (dd, $J_2 = 1.1$ Hz, $J_{gem} = 16.2$ Hz, 2H), 3.62 (s, 2H), 3.72 (dd, $J_3 = 11.3$ Hz, $J_{gem} = 16.2$ Hz, 2H), 5.54 (dd, $J_2 = 1.1$ Hz, $J_3 = 11.3$ Hz, 2H), 6.08 (s, 4H), 6.67–7.33 (m, 12H), 9.40 (s, 2H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 22.1$, 39.7, 40.1, 41.5, 101.6, 115.8, 121.6, 126.5, 128.0, 128.5, 130.1, 132.1, 148.2, 149.4, 152.5, 154.7, 167.6 ppm; EI-MS (*m/z*, %): 660 [M^+] (23), 600 (38), 280 (100) *m/z*. Anal. Calcd. for $C_{37}H_{32}N_4O_8$: C, 67.26; H, 4.88; N, 8.48. Found: C, 67.19; H, 4.80; N, 8.55.

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References

[1] N. Gökhan, A. Yeşilada, G. Uçar, K. Erol, A.A. Bilgin, Arch. Pharm. Med. Chem. 336 (2003) 362–371.
[2] P.G. Baraldi, G. Balboni, M.G. Pavani, G. Spalluto, M.A. Tabrizi, E. De Clercq, J. Balzarini, T. Bando, H. Sugiyama, R. Romagnoli, J. Med. Chem. 44 (2001) 2536–2543.
[3] I. Antonini, P. Palucci, A. Magnano, S. Martelli, J. Med. Chem. 44 (2001) 3329–3333.
[4] S. Hernandez, I. Moreno, R. SanMartin, M.T. Herrero, E. Dominguez, Org. Biomol. Chem. (2011). doi:10.1039/C0OB00812E.
[5] B.S. Holla, P.M. Akbarali, M.K. Shivanada, Il Farmaco 55 (2000) 256–263.
[6] Y.R. Prassad, A.L. Rao, L. Prasoona, K. Murali, P.R. Kumar, Bioorg. Med. Chem. Lett. 15 (2005) 5030–5034.
[7] N. Gökhan-Kelekçi, S. Koyunoğlu, S. Yabanoğlu, K. Yelekçi, Ö. Özgen, G. Uçar, K. Erol, E. Kendi, A. Yeşilada, Bioorg. Med. Chem. 17 (2009) 675–689.
[8] G. Turan-Zitouni, P. Chevallet, F.S. Kiliç, K. Erol, Eur. J. Med. Chem. 35 (2000) 635–641.
[9] O. Bruno, A. Ranise, F. Bondavalli, F. Schenone, M. D'Amico, A. Filipelli, W. Filipelli, F. Rossi, Farmaco 48 (1993) 949–966.
[10] A.R. Bhat, F. Athar, A. Azam, Eur. J. Med. Chem. 44 (2009) 426–431.
[11] B. Insuasty, H. Martinez, J. Quiroga, R. Abonía, M. Nogueras, J. Cobo, J. Heterocycl. Chem. 45 (2008) 1521–1524.
[12] R.P. Ganorkar, V.S. Jamode, Asian J. Chem. 15 (2003) 475–478.
[13] A. Loupy, A. Petit, J. Hamelin, F. Texier-Boullet, P. Jacquault, D. Mathe, Synthesis (1998) 1213–1234.
[14] R.S. Varma, Green Chem. (1999) 43–55.
[15] S. Deshayes, M. Liagre, A. Loupy, J.-L. Luche, A. Petit, Tetrahedron 55 (1999) 10851–10870.
[16] K. Tanaka, F. Toda, Chem. Rev. 100 (2000) 1025–1074.
[17] R.S. Varma, Clean Prod. Process. 1 (1999) 132–147.
[18] R.S. Varma, Pure Appl. Chem. (2001) 193–198.
[19] J. Quiroga, S. Cruz, B. Insuasty, R. Abonía, Heterocycl. Commun. 6 (2000) 275–282.
[20] J. Quiroga, C. Cisneros, B. Insuasty, R. Abonía, M. Nogueras, A. Sánchez, Tetrahedron 42 (2001) 5625–5627.
[21] J. Quiroga, A. Rengifo, B. Insuasty, R. Abonía, M. Nogueras, A. Sánchez, Tetrahedron Lett. 43 (2002) 9061–9063.
[22] A. Nagaraj, C.S. Reddy, J. Heterocycl. Chem. 44 (2007) 1181–1185.
[23] C.S. Marvel, N. Tarköy, J. Am. Chem. Soc. 79 (1957) 6000–6002.
[24] M.R. Boyd, Am. Soc. Cancer. Res. 30 (1989) 652–663.
[25] A.P. Monks, D.A. Scudiero, P. Skehan, R.H. Shoemaker, K.D. Paull, C. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, M. Mayo, M. Boyd, J. Natl. Cancer Inst. 83 (1991) 757–766.
[26] J.N. Weinstein, T.G. Myers, P.M. O'Connor, S.H. Friend Jr., A.J. Fornace, K.W. Kohn, T. Fojo, S.E. Bates, L.V. Rubinstein, N.L. Anderson, J.K. Buolanwini, W.W. van Osdol, A.P. Monks, D.A. Scudiero, E.A. Sausiville, D.W. Zaharevitz, B. Bunow, V.N. Viswanadhan, G.S. Jhonson, R.E. Wittes, K.D. Paull, Science 275 (1997) 343–349.
[27] W.C. Hubbard, M.C. Alley, G.N. Gray, K.C. Green, T.L. McLemore, M.R. Boyd, Cancer Res. 49 (1989) 826–832.