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Note

Synthesis and pharmacological activities of novel furobenzopyrone and benzofuran derivatives

E R El-Sawy*[1], Kamel H Shaker[2], A H Mandour[1], A Shehab El-Din[3] & M M Abdula[4]

[1] Chemistry Department of Natural Compounds, National Research Centre, Dokki, Cairo, Egypt

[2] University of Bayreuth, Organic Chemistry NWII, I/2, Bayreuth, Germany

[3] Drug Radiation Research Departments, National Centre for Radiation Research, Technology, Atomic Energy Authority, Cairo, Egypt

[4] Research Unit, Egyptian Pharmacist Co., Cairo, Egypt

E-mail: ahmandour_z@yahoo.com

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The reactions of 2-cyanoacetohydrazide and 2'-acetyl-2-cyano-acetohydrazide with each of furobenzopyrone derivatives **1a-d** and benzofuran derivatives **10a,b** have been studied. The structures of the new compounds are confirmed from their elemental analyses and spectral data. Also, the antimicrobial and antiinflammatory activity of the new compounds has been evaluated.

Keywords: Furobenzopyrone, benzofuran, pyrazolone, pyrazolidine, photo biological activity, anti-inflammatory effect

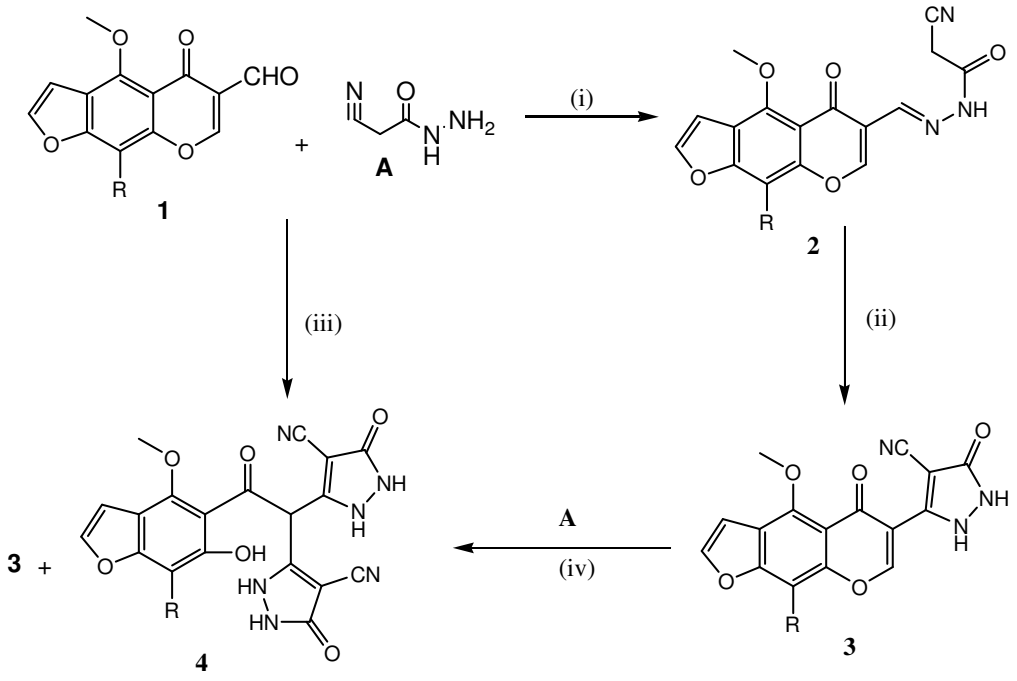
Furobenzopyrone derivatives occupy a position of considerable significance for widespread occurrence in plants and their potential as important pharmaceuticals[1-3]. It has also been reported that benzofuran derivatives possess bacteriostatic, bactericidal, fungistatic and fungicidal activities[4-10]. On the other hand, pyrazoline, pyrazolidine and bicyclic pyrazolines are known for their pronounced antimicrobial activity[11-17]. Furthermore, pyrazolines which contain furobenzopyrone or benzofuran moiety are well known to possess broad spectrum anti-inflammatory activity[18-21]. Therefore, the goal of this work is the synthesis of new furobenzopyrone and benzofuran containing uncondensed pyrazolone and pyrazolidine moieties and evaluating their antimicrobial as well as anti-inflammatory effects.

Results and Discussion

In the present work, 6-formyl visnagin **1a** or 6-formyl khellin **1b** was condensed separately with 2-cyanoacetohydrazide **A** in 1:1 molar ratio under stirring at RT in 95% acetic acid to yield 6-(2-cyano-N'-methylidene acetohydrazido) furobenzopyrones **2a** and **2b,** respectively (**Scheme I**). IR spectra of **2a,b** showed characteristic absorption bands at 2200 cm^{-1} (CN) and at 3200 and 3218 cm^{-1}(NH) groups. Their ^{1}H NMR (DMSO-d_6) spectra revealed singlet at δ 8.51 attributed to anil proton (CH=N) for each.

It is previously reported that[22] α-cyanoacetyl aldehyde hydrazones cyclize in acetic acid (95%) and yield the corresponding pyrazolone-4- carbonitrile. In the present work and under the reported conditions, compounds **2a** and **2b** yielded the 6-(pyrazolone-4-carbonitrile)furobenzopyrone derivatives **3a** and **3b**, respectively (**Scheme I**). The IR spectra showed strong absorption bands at 2200 and 2219 cm^{-1} (CN) and at 1700 and 1670 cm^{-1} (C=O) for **3a** and **3b** respectively. The mass spectra showed molecular ion peak at *m/z* 323 and 353 for **3a** and **3b** respectively. Moreover, ^{1}H NMR (MeOD) spectra lack the presence of the anil protons detected in the parent spectra of **2a,b** and revealed two singlet signals (D_2O exchangeable) at δ 7.92 and 4.91 for (NHs) besides the methoxy and aromatic protons which are located at their positions. ^{13}C NMR (MeOD) spectra revealed signals at δ 170.3 and 173.1 (keto groups) and 115.5 (nitrile groups).

On the other hand, condensation of compound **1a** with **A** in 1:2 molar ratio in refluxing 95% acetic acid yielded a product with m.p. 134-6°C assigned as **3a** together with another one with m.p. 171-2°C separated from the mother liquor which was assigned as **4a**. Compound **3a** gave negative ferric chloride test while compound **4a** gave positive ferric chloride test. Also, the postulated structure of **4a** was assigned based on its analytical and spectral data (**Tables I** and **II**), and also comparing with the literature[2,23,24] values. Similarly, condensation of **1b** with **A** in 1:2 molar ratio and under the above mentioned conditions gave **3b** and **4b** (**Scheme I**). IR spectrum of compound **4a,** for example, showed absorption bands at 3400 (OH), 3220 and 3200 (NH), 2200 (CN) and 1700 and 1689 cm^{-1} (keto groups). Its mass spectrum showed molecular ion peak at *m/z* 420. ^{1}H NMR (MeOD) revealed three separate singlets (D_2O exchangeable) at δ 11.72 (phenolic OH) and



a, R = H
b, R = OCH_3

Scheme I — Reagents and conditions: (i) stirring in 95% acetic acid in 1:1 molar ratio, RT, (ii) reflux in 95% acetic acid, (iii) in 95% acetic acid in 1:2 molar ratio, (iv) reflux in 95% acetic acid

7.91 and 4.92 (NHs). ^{13}C NMR (MeOD) spectrum of **4a** revealed signals at δ 192.1 for the free keto group and absence of the cyclic keto carbon of γ–pyrone as compared **3a** with δ 182.2 (CONH) and 117.1 (CN) (**Figure 1**). Based on these data, the assigned structure **4a** was proposed as 4-methoxy-6-hydroxy-5-[(1-oxo-ethan-2-yl)-2,2-bis(2,3-dihydro-3-oxo-4-cyano-1*H*-pyrazol-5-yl) benzofuran (**Scheme I**).

Moreover, compounds **4a,b** could be obtained in good yields *via* the reaction of 2-cyanoacetohydrazide **A** with compounds **3a,b** in refluxing 95% acetic acid . Compounds **4a,b** which were obtained by the two routes showed no depression in admixed melting points.

Formation of the bis-pyrazolone derivatives **4a,b** may be proceeded firstly *via* the hydrazone formation including γ-pyrone ring fission[2,23,24], followed by intramolecular cyclization and loss of hydrogen molecule.

Parallel results were obtained when **1c** and/or **1d** was condensed with **A** in molar ratio 1:1 at RT and gave 4-methoxy **5a** and 4,9-dimethoxy **5b**–5–oxo –7–methyl–6-(2-cyano-N'-ethylidene acetohydrazido) furo(3,2-g)benzopyrans (**Scheme II**). But at 1:2 molar ratio in refluxing acetic acid, the final products were assigned as pyrazolidine and not 2,3-dihydropyrazole namely, 4-methoxy **6a** and 4,9-dimethoxy **6b** -5-oxo-7-methyl-6-(3–oxo–4– cyano – 5–methyl–pyrazolidin-5-yl) furo(3,2-g)benzopyranes and 4-methoxy **7a** and 4,9-dimethoxy **7b** -6-hydroxy-5- [(1–oxo–ethan–2– yl)-2,2- bis (3-oxo- 4-cyano-5-methyl-pyrazolidin-5-yl)] benzofurans (**Scheme II**), based on their analytical and spectral data (**Tables I** and **II**)

Base catalyzed reaction (Knoevenagel reaction) of compounds **1a** and/or **1b** with 2'-acetyl-2-cyanoacetohydrazide **B** yielded the pyrazolinone derivatives **8a** and **8b** in good yields (**Scheme III**). The structures of **8a,b** were confirmed based on their elemental analyses and spectral data . IR spectrum of compound **8a** for example showed characteristic absorption bands at 3218(NH), 2219(CN) and 1710 and 1699 cm^{-1} (keto groups). Its mass spectrum showed molecular ion peak at *m/z* 365. Its ^{1}H NMR (DMSO-d_6) revealed a singlet at δ 2.02 (3H, s, $COCH_3$) besides the methoxy and aromatic protons which were located at their positions.

On the other hand, compounds **1c** and / or **1d** condensed with **B** under the above mentioned conditions and gave products **9a,b** (**Scheme III**).

Table I — Physical and analytical characterization data of the prepared compounds

Compd	Mol. formula (Mol. Wt.)	m.p. (°C)	Yield %	Calcd % (found)		
				C	H	N
2a	$C_{16}H_{11}N_3O_5$ 325	82-4	65 yellow	59.07 (59.00	3.38 3.21	12.92 13.10)
2b	$C_{17}H_{13}N_3O_6$ 355	162-4	55 yellow	57.46 (57.50	3.66 3.70	11.83 12.00)
3a	$C_{16}H_9N_3O_5$ 323	134-6	45 buff	59.44 (59.22	2.78 2.81	13.00 12.89)
3b	$C_{17}H_{11}N_3O_6$ 353	168-170	45 orange	57.79 (57.80	3.68 3.55	11.89 11.70)
4a	$C_{19}H_{12}N_6O_6$ 420	171-3	30 yellow	54.28 (54.11	2.85 2.59	20.00 20.02)
4b	$C_{20}H_{14}N_6O_7$ 450	60-2	30 brown	53.33 (53.12	3.11 3.00	18.66 18.59)
5a	$C_{17}H_{15}N_3O_5$ 341	93-5	54 yellow	59.82 (60.00	4.39 4.22	12.31 12.22)
5b	$C_{18}H_{17}N_3O_6$ 371	126-8	56 yellow	58.22 (58.44	4.58 3.69	11.32 11.22)
6a	$C_{18}H_{15}N_3O_5$ 353	99-101	yellow	61.18 (61.00	4.24 4.20	11.89 11.95)
6b	$C_{19}H_{17}N_3O_6$ 383	74-6	yellow	59.53 (59.44	4.43 4.32	10.96 11.00)
7a	$C_{21}H_{20}N_6O_6$ 452	199-201	orange	55.75 (55.67	4.42 4.32	18.58 18.44)
7b	$C_{22}H_{22}N_6O_7$ 482	101-2	brownish	54.77 (54.63	4.14 4.00	17.42 17.50)
8a	$C_{18}H_{11}N_3O_6$ 365	110-2	75 orange	59.17 (59.02	3.01 3.00	11.50 11.25)
8b	$C_{19}H_{13}N_3O_7$ 395	90-2	70 reddish brown	57.72 (57.65	3.29 3.11	10.63 10.55)
9a	$C_{20}H_{17}N_3O_6$ 395	95-7	71 yellow	60.75 (60.59	4.30 4.22	10.63 10.55)
9b	$C_{21}H_{19}N_3O_7$ 425	81-3	70 pistachio	59.29 (59.02	4.47 4.39	9.88 9.75)
13a	$C_{14}H_{13}N_3O_4$ 287	100-2	70 yellow	58.53 (58.45	4.52 4.44	14.63 14.50)
13b	$C_{15}H_{15}N_3O_5$ 317	122-4	72 yellow	56.78 (56.55	4.73 4.65	13.24 13.02)
14a	$C_{16}H_{15}N_3O_5$ 329	93-5	75 yellow	58.35 (58.22	4.55 4.35	12.76 12.65)
14b	$C_{17}H_{17}N_3O_6$ 359	95-7	71 pistachio	56.82 (56.55	4.73 4.65	11.69 11.55)

Their ^{1}H NMR (DMSO-d_6) spectra showed singlet at δ 4.11 attributed to CH-CN besides the acetyl, methoxy and aromatic protons which are located at their positions. Mass spectra showed molecular ion peaks at *m/z* 395 and 425, respectively.

Formation of pyrazolines **8a,b** and pyrazolidine **9a,b** were formed with the condensation of the active methylene group of **B** with the free keto group of compounds **1a-d** forming the yildene compounds followed by intramolecular cyclization.

Condensation of visnaginone **10a** and/or khellinone **10b** with 2-cyanoacetohydrazid **A** in refluxing 95% acetic acid led to the formation of 5-(pyrazolidine)benzofurans derivatives **13a,b**

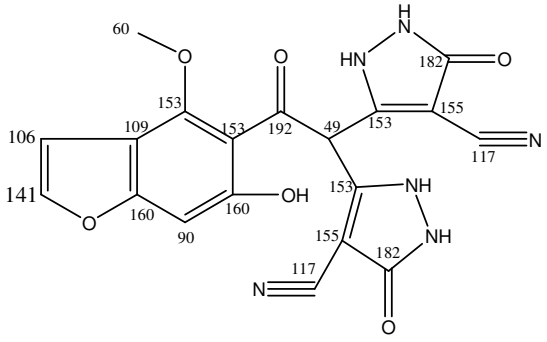
Table II—Spectral characterization of the prepared compounds

Compd	NMR (δ, ppm)		MS
	^{1}H	^{13}C	*m/z* (%)
2a	(DMSO-d_6) 8.51(1H, s, CH=N), 7.56 (1H, d, H-2), 7.01(1H, s, H-7), 6.62(1H, d, H-3), 6.07(1H, s, H-9), 4.95(1H, s, NH), 4.22(2H, s, CH_2), 3.91(3H, s, OCH_3)		
2b	(DMSO-d_6) 8.51(1H, s, CH=N), 7.57 (1H, d, H-2), 7.07(1H, s, H-7), 6.93(1H, d, H-3), 4.97(1H, s, NH), 4.22(2H, s, CH_2), 3.84 & 3.91(6H, 2s, $2OCH_3$)		
3a	(MeOD) 7.92(1H, s, NH), 7.56(1H, d, H-2), 7.07(1H, s, H-7), 6.66(1H, d, H-3), 6.56(1H, s, H-9), 4.91(1H, s, NH), 3.91(3H, s, OCH_3)	(MeOD) 61.1(OCH_3), 115.5(CN), 90.7-152.2(Ar-C), 170.3&173.1 (C=O)	323(M^+,10),216(100), 168(61), 107(3)
3b	(MeOD) 10.21 (1H, s, NH), 7.88(1H, d, H-2), 7.21(1H, s, H-7), 7.01(1H, d, H-3), 4.39(1H, s, NH), 3.88 &3.91(6H, 2s, $2OCH_3$)	(MeOD) 64.25(OCH_3), 115.5 (CN), 106.1-148.8(Ar-C), 170.8-161.9 (C=O)	353(M^+,20), 246 (100), 186(50), 107 (5)
4a	(MeOD) 11.72(1H, s, OH) 7.91 (2H, s, 2NH), 7.56(1H, d, H-2), 6.99(1H, s, H-7), 6.76(1H, d, H-3), 4.92(2H, s, 2NH), 3.85(3H, s, OCH_3), 2.64(1H, s, CH)	(MeOD) 49.1(-CH-), 61.1(OCH_3), 117.1(CN), 106.1-152.7(Ar-C), 160.9 (C-OH), 182.2(CONH), 192.7(C=O)	420(M^+,10), 226(28), 191(100), 163(70)
4b	(MeOD) 9.33(1H, s, OH) 7.99 (4H, s, 4NH), 7.81(1H, d, H-2), 7.01(1H, d, H-3), 3.85 & 3.91(6H, 2s, $2OCH_3$), 2.62(1H, s, CH)	(MeOD) 32.1(-CH-), 61.4(OCH_3), 118.1(CN), 105.1-148.1(Ar-C), 165.1 (C-OH), 180.2(CONH), 187.6(C=O)	450(M^+,10), 226(30), 221(100), 193(30)
5a	(DMSO-d_6) 7.56 (1H, d, H-2), 6.90(1H, d, H-3), 6.61(1H, s, H-9), 4.95(1H, s, NH), 4.22(2H, s, CH_2), 3.91(3H, s, OCH_3), 1.91(6H, s, 2 CH_3)		
5b	(DMSO-d_6) 7.51 (1H, d, H-2), 6.90(1H, d, H-3), 4.90(1H, s, NH), 4.22(2H, s, CH_2), 3.84& 3.91(6H, 2s, $2OCH_3$), 1.91(6H, s, 2 CH_3)		
6a	(DMSO-d_6) 7.92(1H, s, NH), 7.52 (1H, d, H-2), 6.90(1H, d, H-3), 6.61(1H, s, H-9), 4.95(1H, s, NH), 4..12(H, s, CH), 3.87(3H, s, OCH_3), 1.91(6H, s, $2CH_3$)	(DMSO-d_6) 23.5(CH_3), 32.0(-CH-), 61.4(OCH_3), 115.1(CN), 106.1-152.1(Ar-C), 170.3&161.6(C=O)	353(M^+, 30), 230 (100), 202 (4), 122 (10)
6b	(DMSO-d_6) 7.90(1H, s, NH), 7.52 (1H, d, H-2), 6.90(1H, d, H-3), 4.93(1H, s, NH), 4.12(H, s, CH), 3.87&3.91(6H, 2s, $2OCH_3$), 1.92(6H, s, $2CH_3$	(DMSO-d_6) 23.1(CH_3), 32.0(-CH-), 61.4(OCH_3), 115.3(CN), 106.1-152.1(Ar-H), 157.1 (C-OH), 170.3 &162.6(C=O)	383 (M^+, 20), 260 (100), 232 (5), 122(9)
7a	(DMSO-d_6) 11.77(1H, s, OH) 10.01&11.25(4H, 2s, 4NH), 7.56 (1H, d, H-2), 7.11(1H, s, H-7), 6.67(1H, d, H-3), 4.12(2H, s, 2CH-CN), 3.85(3H, s, OCH_3), 3.21(1H, s, CH), 1.91(6H, s, $2CH_3$)	(DMSO-d_6) 23.1(CH_3), 32.0(-CH-), 46.2(-CH-),61.4(OCH_3), 115.3 (CN), 106.1-152.1(Ar-C), 158.1 (C-OH), 170.3 & 167.6(CONH), 203.1(C=O)	452(M^+, 10), 258(40), 191(100), 163(40)
7b	(DMSO-d_6) 11.78(1H, s, OH) 10.01&11.21(4H, 2s, 4NH), 7.56 (1H, d, H-2), 6.91(1H, d, H-3), 4.12(2H, s, 2CH-CN), 3.85& 3.91(6H, 2s, $2OCH_3$), 3.21(1H, s, CH), 1.62(6H, s, $2CH_3$)	DMSO-d_6) 23.2(CH_3), 32.0(-CH-), 46.2(-CH-),61.4(OCH_3), 115.1 (CN), 106.3-152.7(Ar-C), 158.1 (C-OH), 170.9 & 167.6(CONH), 203.0(C=O)	482(M^+, 10),288(39), 221(100), 193(30)
8a	(DMSO-d_6) 11.21(1H, s, NH), 7.52 (1H, d, H-2), 7.20(1H, s, H-7), 7.01(1H, d, H-3), 6.61(1H, s, H-9), 3.82(3H, s, OCH_3), 2.02 (3H, s, $COCH_3$)	DMSO-d_6) 30.2(CH_3), 61.4 (OCH_3), 117.1 (CN), 96.7-155.7(Ar-C), 162.1, 170.1 & 173.1(C=O)	365(M^+, 30), 215 (100), 187(30), 151 (80)
8b	(DMSO-d_6) 11.70(1H, s, NH), 7.53 (1H, d, H-2), 7.01(1H, s, H-7), 6.71(1H, d, H-3), 3.82 & 3.91(6H, 2s, $2OCH_3$), 2.02 (3H, s, $COCH_3$)		395(M^+, 20), 245 (100), 217(20), 151 (75)

—Contd

Table II—Spectral characterization of the prepared compounds—*Contd*

Compd	NMR (δ, ppm)		MS
	^{1}H	^{13}C	*m/z* (%)
9a	(DMSO-d_6) 10.21(1H, s, NH), 7.53 (1H, d, H-2), 6.90(1H, d, H-3), 6.61(1H, s, H-9), 4.11(1H, s, CH), 3.82 (3H, s, OCH_3), 2.01(3H, s, $COCH_3$), 1.65(6H, s, 2 CH_3)	DMSO-d_6) 23.2& 32.0 (CH_3), 61.4(OCH_3), 117.1 (CN), 99.3-155.7(Ar-C), 168.2&173.2(C=O)	395(M^+, 20), 230(100), 202(5), 165(87)
9b	(DMSO-d_6) 10.00(1H, s, NH), 7.53 (1H, d, H-2), 6.93(1H, d, H-3), 4.11(1H, s, CH), 3.82 & 3.91 (6H, 2s, 2OCH_3), 2.01(3H, s, $COCH_3$), 1.91(6H, s, 2 CH_3)		425(M^+, 10), 260(100), 232(4), 165(83)
13a	(DMSO-d_6) 12.52(1H, s, OH) 10.02&11.21(2H, 2s, 2NH), 7.56 (1H, d, H-2), 7.21(1H,s, H-7), 7.01(1H, d, H-3), 4.12(1H, s, CH), 3.85(3H, s, OCH_3), 1.61 (3H, s, CH_3)	DMSO-d_6) 20.2 (CH_3), 30.1 (=C=), 60.5(OCH_3), 117.1 (CN), 91.7-153.5(Ar-C), 157(C-OH), 168.1 (C=O)	287(M^+, 10), 178(100), 146(20), 111(2)
13b	(DMSO-d_6) 11.88(1H, s, OH) 10.07&11.29(2H, 2s, 2NH), 7.56 (1H, d, H-2), 6.67(1H, d, H-3), 4.12(1H, s, CH), 3.85 & 3.91(6H, 2s, 2OCH_3), 1.69 (3H, s, CH_3)		317(M^+, 10), 206(100), 176(20), 111(20)
14a	(DMSO-d_6) 11.82(1H, s, OH), 10.22(1H, s, NH), 7.53 (1H, d, H-2), 6.98(1H, d, H-7), 6.66(1H,s, H-3),4.12(1H, s, CH), 3.82 (3H, s, OCH_3), 2.32(3H, s, $COCH_3$), 1.61(6H, s, 2 CH_3)	DMSO-d_6) 20.2& 23.09 (CH_3), 30.0(=C=), 60.9(OCH_3), 115.1 (CN), 91.8-153.7(Ar-C), 158.1(C-OH), 168.2 (C=O)	329(M^+, 10), 176(100), 149(20)
14b	(DMSO-d_6) 11.70(1H, s, OH), 10.21(1H, s, NH), 7.61 (1H, d, H-2), 6.91(1H, d, H-3), 4.12(1H, s, CH), 3.82 & 3.91 (6H, 2s, 2OCH_3), 2.41(3H, s, $COCH_3$), 1.61(6H, s, 2 CH_3)		359((M$^+$, 10), 206 (100), 176(2), 149 (16)



4a

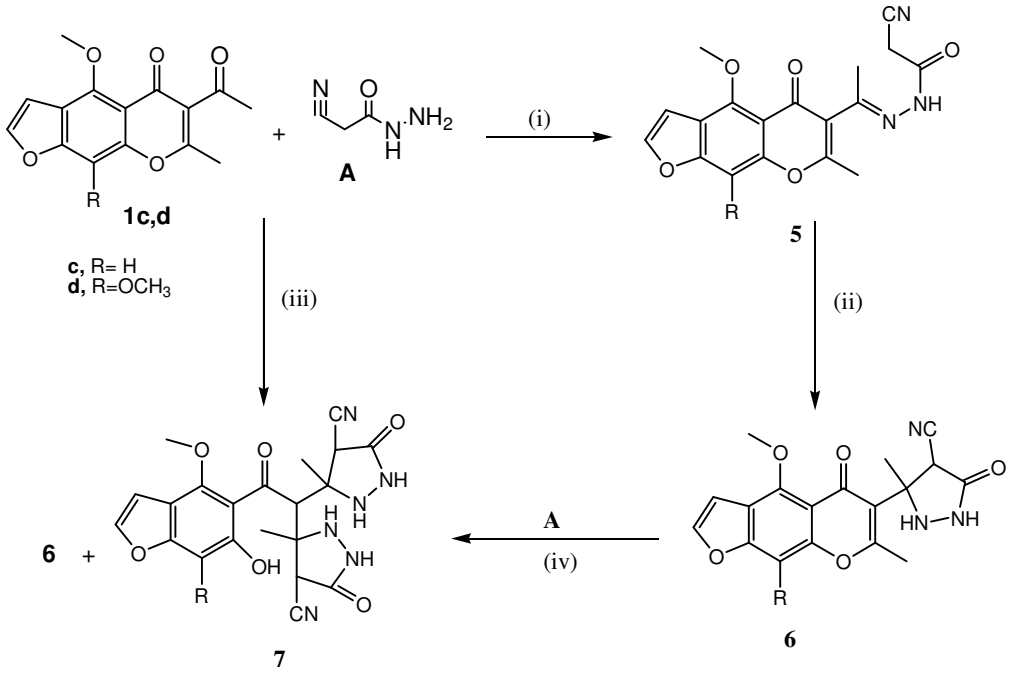
Figure 1

(**Scheme IV**). Analytical and spectral data (**Tables I** and **II**) were in complete agreement with the structure of **13a,b**. For example, IR spectrum of compound **13a** showed characteristic absorption bands at 3450 (OH), 3218 (NH, br), 2219 (CN) and 1687 cm^{-1} (keto groups). ^{1}H NMR (DMSO-d_6) revealed two separate singlets at δ 1.61 (CH_3) and 4.12 (CH), and (D_2O, exchangeable), three singlet signals at 12.52(OH), 11.21 and 10.02 (NHs). ^{13}C NMR (DMSO-d_6) spectrum of **13a** revealed signals at δ 168.1 (cyclic keto groups) and 115.1 (CN).

On the other hand, condensation of visnaginone **10a** and / or khellinone **10b** with 2'-acetyl-2-cyanoacetohydrazide **B** in refluxing dioxane and in the presence of organic base (Knoevenagel reaction) gave 5-(acetyl pyrazolidine)benzofurans derivatives **14a,b** (**Scheme IV**). Compound **14a** showed characteristic absorption bands at 3400 (OH), 3200 (NH), 2200 (CN) and 1687 cm^{-1} (keto groups). ^{1}H NMR (DMSO-d_6) revealed three singlet signals at δ 1.61 (CH_3), 2.32 ($COCH_3$) and 4.12 (CH) and two singlets at δ 11.82 (OH) and 10.22 (NH) (D_2O, exchangeable). The formation of the target compounds **13a,b** and **14a,b** may proceed initially *via* the formation the hydrazones **11a,b** and yildenes **12a,b** (unseparated) followed by intramolecular cyclization.

Antimicrobial Activity

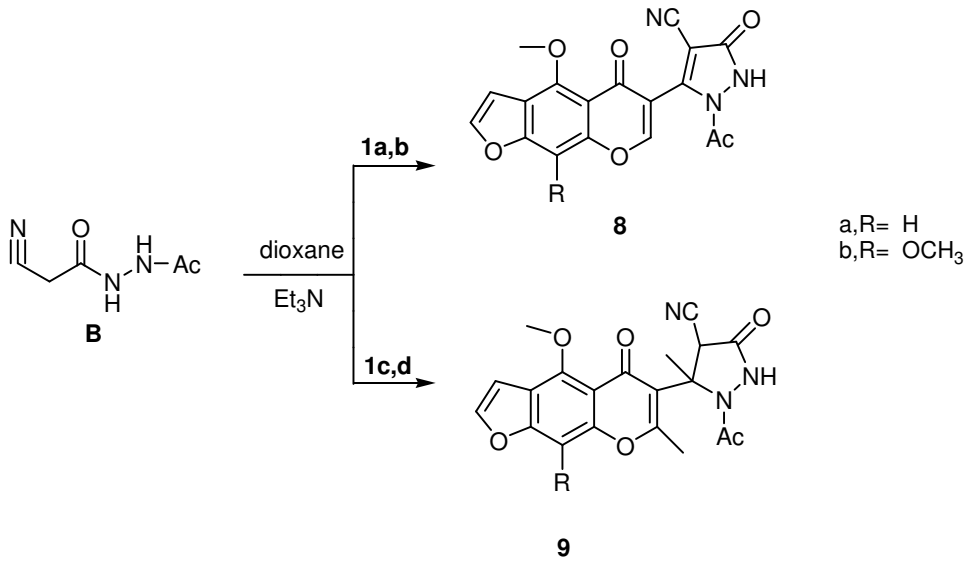
Four bacterial isolates representing Gram-positive and Gram-negative , *Staphylococcus aureus*, *Esherichia coli*, *Klebseilla pneumoniae*, and *Pseudomonas aeruginosa* were recovered on Nutrient and MacConky agar. One tests fungus *Candida albicans* was isolated on Sabouraud Dextrose agar



a, R = H

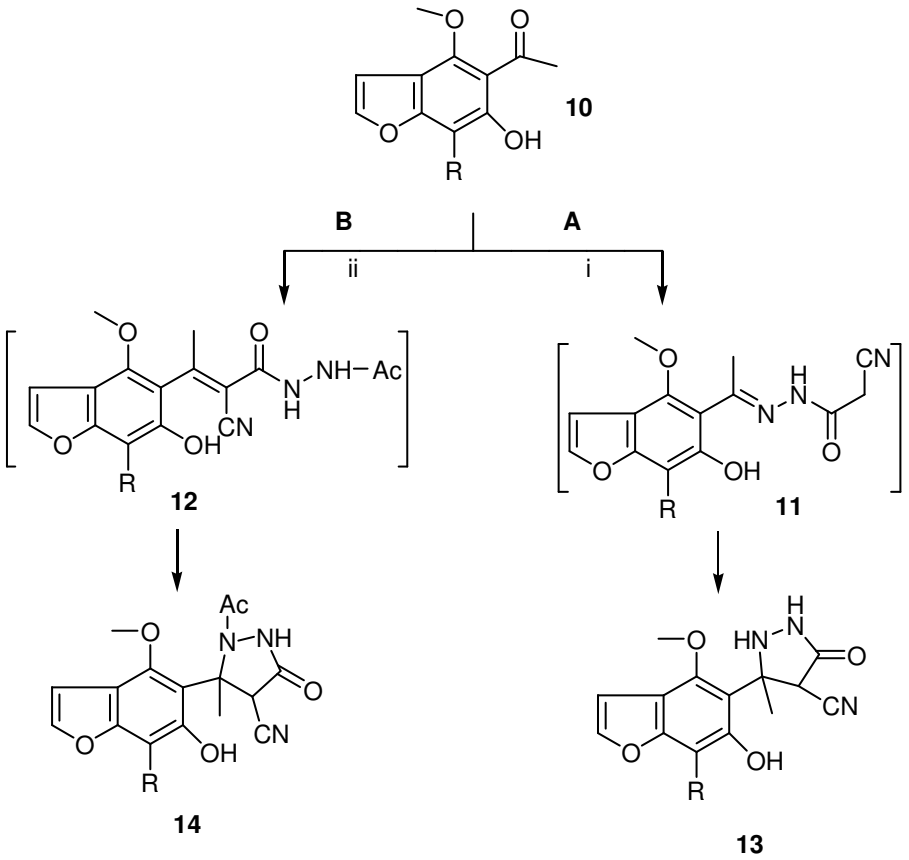
b, R = OCH_3

Scheme II — Reagents and conditions: (i) stirring in 95% acetic acid in 1:1 molar ratio, RT, (ii) reflux in 95% acetic acid, (iii) in 95% acetic acid in 1:2 molar ratio, (iv) reflux in 95% acetic acid



Scheme III

(Oxoid). They were isolated from clinical samples and identified to the species level according to different API systems (BioMerieux). Most of the prepared compounds were tested *in vitro* using the agar diffusion disk method[25,26]. Antimicrobial potentialities of the tested compounds were estimated by placing presterilized filter paper disks (6 mm in diameter) impregnated with 50 µg/ disk. DMF which showed no inhibition zones was used as a solvent. Inhibition zones (IZ) of the tested compounds (mm) were measured after 24-48 hr incubation at 37°C for bacteria and after 5 days incubation at 28°C for fungus (**Table III**). Also, the antibacterial activity of the prepared compounds was tested against each of



Scheme IV — Reagents and conditions: (i) 95% *Ac*OH, reflux, (ii) dioxane, reflux, Et_3N

Gram-positive *S. aureus* and Gram-negative *E. coli* using similar procedures but the Petri-dishes containing the microorganism and the impregnated disks were exposed to UV light (366 nm) for 3 hr before incubation. The obtained data are shown in **Table IV**. Moreover, the minimal inhibitory concentrations (MIC) of the biologically active compounds **6a,b, 8a,b, 9a,b, 13a,b** and **14a,b** (**Table V**) on Gram-negative bacteria *E. coli* was applied using different concentrations. Reference antibiotics disks (6 mm in diameter) supplied by Bristol Myers Squib, Giza, Egypt, Piperacillin (PIP), Amikacin (AN) and fungicide Mycostatin (30 U) were used for comparison.

From the data obtained (**Table III**) it is obvious that all the compounds under test showed moderate to high activity towards Gram-ve bacteria *E. coli*, while these compounds showed slight to moderate activity towards G+ve bacteria *S. aureus*, compared to the reference drug. On the other hand, all the tested compounds showed slight to non activity towards the fungus *C. albicans*. Moreover, the activity of these compounds increased by exposure to UV light (366 nm) towards each of bacteria and fungus and especially to the fungus (**Table IV**). These phenomena may be attributed to the presence of oxygen and nitrogen atoms which bear free lone pairs of electrons. Also, the MIC of the biological active compounds **6a,b, 8a,b, 9a,b, 13a,b** and **14a,b** was determined (**Table IV**)

Anti-inflammatory Activity

Determination Of Acute Toxicity (ALD_{50})

Albino mice of either sex weighing 20-25 g were used to study the toxicological effects of the chosen compounds. Animals were infected with different increasing doses and then observed within 24 hr for any mortality and then calculated the dose that killed 50% of the animals following the method of Austen and Broklehurst[27]

Table III — Preliminary *in-vitro* antimicrobial activity screening for the synthesized compounds

Compd	Microorganism / inhibition zones (mm)				
	1	*2*	*3*	*4*	*5*
3a	8	10	10	10	8
3b	7	10	10	9	8
4a	7	10	10	9	9
4b	7	10	10	8	9
6a	8	13	7	8	10
6b	8	13	7	8	12
7a	7	10	7	8	8
7b	7	10	8	7	8
8a	12	14	8	7	8
8b	11	15	7	7	8
9a	10	15	7	7	8
9b	10	15	7	8	8
13a	8	15	12	8	7
13b	8	14	12	8	10
14a	12	12	12	7	10
14b	10	15	12	7	10
Amikacin	21	24	20	19	-
Piperacillin	23	25	20	21	-
Mycostatin (30U)	-	-	-	-	25

1: Staphylococcus aureus
2: Escherichia coli
3: Klebseilla pneumonia
4: Pseudomonas aeruginosa
5: Candida albicans
Highly active = Inhibition zone ≥ 12 mm
Moderately active = Inhibition zone ≥ 8- 12 mm
Slightly active = Inhibition zone < 8 mm
Non active = Inhibition zone 6 mm

Table IV — The *in-vitro* antimicrobial activity test for the prepared compounds using the UV light (366 nm)

Compd	Microorganism / IZ (mm)		
	1	*2*	*3*
3a	9	10	10
3b	8	10	10
4a	8	11	11
4b	8	11	11
6a	10	15	9
6b	10	14	9
7a	9	11	9
7b	9	11	9
8a	13	15	9
8b	13	17	11
9a	12	17	12
9b	12	18	13
13a	10	18	12
13b	10	18	10
14a	14	18	10
14b	14	18	11
Amikacin	21	24	-
Piperacillin	23	25	-
Mycostatin	-	-	25

1: Saphylococcus aureus
2: Escherichia coli
3: Candida albicans

Table V — MIC of the highly biological active compounds towards *Escherichia coli*

Compd	Inhibition zones (mm) Conc. µg / disk					
	50	40	30	20	10	5
6a	13	13	11	9	7	-
6b	13	13	9	-	-	-
8a	14	12	10	8	-	-
8b	15	12	10	8	-	-
9a	15	13	12	10	8	-
9b	15	14	12	9	7	-
13a	15	15	13	11	8	-
13b	14	14	13	11	8	-
14a	14	12	12	9	7	-
14b	15	13	12	10	7	-

Evaluation of Anti-inflammatory Activity

The synthesized compounds were tested for their anti-inflammatory activity using carrageanan induced rat hind paw edema method of Winter, *et al*.[28,29] Albino rats of either sex weighting between 150-180 g were divided into eight groups of animals, each of them were orally dosed with tested compounds at a dose level of 25-50 mg/kg 1hr before carrageanan challenge foot paw edema was induced by subplanter injection of 0.05 mL of 1% suspension of carrageanan in saline into the planter tissue of one hind paw. An equal volume of saline was injected into the other hand paw and served as control. 4hr after drug administration, the animals were decapitated, blood was collected and the paws were rapidly excised. The average weight of edema was examined for the tested as well as the control group and the percentage inhibition of weight of edema is calculated according to

$$\% \text{ Inhibition} = \frac{\text{Wt. of paw edema of control - Wt.of paw edema of treated}}{\text{Wt.of paws edema of control}} \times 100$$

Indomethacin was used as the reference drug for comparison.

Estimation of Plasma Prostaglandin E_2 (PGE_2)[30-32]

Heparinized blood samples were collected from rats (n=8), plasma was separated by centrifugation at 12000 g for 2 min at 4°C and immediately stored frozen (-20°C) until use. The designs correlate-EIA prostaglandin in E_2 (PGE_2) kit is a competitive immune assay for the quantitative determination of PGE_2 in biological fluids. The kit uses a monoclonal antibody to PGE_2 in the sample. After a simultaneous incubation at RT the excess reagents were washed away and the substrate was added. After a short incubation time the enzyme reaction was stopped and the yellow color generated was read on a micro-plate reader (DYNATCH, MR 5000) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE_2 in either standard or samples.

Experimental Section

Melting points were determined in open capillary tubes on electrothermal digital melting point (Buchi) and are uncorrected. IR spectra were recorded on a Burker Vector 22 infrared spectrometer using KBr disk. The ^1H and ^{13}C NMR spectra were recorded on Bruker spectrometer (300 and 125 MHz) in DMSO-d_6 and MeOD, using TMS as an internal standard, chemical shift are expressed in δ, ppm (Organic Chemistry Department NWII, I/2, Bayreuth University, Germany). Mass spectra were recorded on JEOL-JMS-AX500 mass spectrometer. Elemental microanalyses were performed at micro-analytical center of Faculty of Science, Cairo University. The homogeneity of the compounds was confirmed by thin layer chromatography using silica gel $60F_{254}$ and spots were located by exposure to iodine vapours.

Visnagin and khellin were purchased from Chemical Industrial Developing Company (CID), Giza, Egypt. The starting materials visnaginone **10a** and khellinone **10b** were prepared *via* alkaline hydrolysis of the naturally occurring visnagin[33] and khellin[34]. Vilsmeier-Haack reaction of compounds **10a,b** yielded 6-formyl visnagin **1a** (Ref.35) and 6-formyl khellin **1b** (Ref.35). Treatment of **10a,b** with acetic acid in the presence of sodium acetate yielded 3-acetyl visnagin **1c** (Ref.36) and 3-acetyl khellin **1d** (Ref.36). 2-Cyanoacetohydrazide **A** and 2'-acetyl-2-cyanoacetohydrazide **B** were prepared as reported[37,38].

Preparation of 4-methoxy (2a) and 4,9-dimethoxy(2b)-5-oxo-6-[(2-cyano-N'-methylidene) acetohydrazido] furo(3,2-g)benzopyranes

To a solution of compound **1a** or **1b** (0.01 mol) in acetic acid (95%, 10 mL), was added 2-cyanoacetohydrazide **A** (0.99 g, 0.01 mol). The reaction mixture was stirred at RT for 1 hr. The solid product that formed was filtered off, washed with water, air dried and purified by recrystallization from aqueous ethanol.

Cyclization of compounds 2a,b

Preparation of 4-methoxy (3a) and 4,9-dimethoxy (3b)-5-oxo-6-(2,3-dihydro-3-oxo-4-cyano-1*H*-pyrazol-5-yl)furo(3,2-g)benzopyranes

Compound **2a** or **2b** (0.01 mol) was heated under reflux in acetic acid (95%, 10 mL) for 5 hr. The reaction mixture was then evaporated to half of its volume under reduced pressure and left to cool in a refrigerator. The product that was obtained was collected by filtration, washed with water, air dried and purified by recrystallization from aqueous ethanol.

Preparation of 4-methoxy (4a) and 4,9-dimethoxy (4b)-6-hydroxy-5-[(1-oxo-ethan-2-yl)-2,2-bis(2,3-dihydro-3-oxo-4-cyano-1*H*-pyrazol-5-yl)] benzofurans

Method A

A mixture of compound **1a** or **1b** (0.01 mol) and 2-cyanoacetohydrazide **A** (1.98 g, 0.02 mol) in acetic acid (95%, 10 mL) was refluxed for 3 hr, After cooling (5°C**),** the formed solid product was filtered off, washed with water, air dried and purified by recrystallization from aqueous ethanol to give **3a** or **3b** with yield ~ 54%. The filtrate was concentrated under reduced pressure to half of its volume and water (50 mL) was added. The separated solid was filtered off, washed with water, air dried and purified by recrystallization from diethyl ether to give **4a,b** (**Table I**).

Method B

A mixture of compound **2a** or **2b** (0.01 mol) and 2-cyanoacetohydrazide **A** (0.99 g, 0.01 mol) in acetic acid (95%, 10 mL) was refluxed for 2 hr. The reaction mixture was then concentrated under reduced pressure to half of its volume and water was added (50 mL). The formed product was filtered off, washed with water, air dried and purified by recrystallization from diethyl ether.

Preparation of 4-methoxy (5a) and 4,9-dimethoxy (5b) -5-oxo-7-methyl-6-[(2-cyano-N'-ethylidene) acetohydrazido] furo (3,2-g) benzopyranes

Compounds **5a,b** were prepared through the reaction of compound **1c** or **1d** with **A** as mentioned before for the preparation of compounds **2a,b**

Preparation of 4-methoxy (6a) and 4,9-dimethoxy (6b) -5-oxo-7-methyl-6-(3-oxo-4-cyano-5-methyl-pyrazolidin-5-yl) furo (3,2-g) benzopyranes

Compounds **6a,b** were prepared through the reaction of compound **5a** or **5b** with **A** as mentioned before for the preparation of compounds **3a,b**

Preparation of 4-methoxy (7a) and 4,9-dimethoxy (7b) -6-hydroxy-5-[(1-oxo-ethan-2-yl) -2,2- bis (3-oxo-4-cyano-5-methyl-pyrazolidin-5-yl)] benzofurans

Compounds **7a,b** were prepared through the reaction of compound **1c** or **1d** with **A** as mentioned before for the preparation of compounds **4a,b**

Preparation of 4-methoxy (8a) and 4,9-dimethoxy (8b) -5-oxo-7-methyl -6-(1-acetyl-3-oxo-4-cyano-5-methyl-pyrazolidin-5-yl) furo (3,2-g) benzopyranes

To a solution of 2'-acetyl-2-cyanoacetohydrazide **B** (1.41 g, 0.01 mol) in dioxane (15 mL) and triethylamine (1 mL), compound **1a** or **1b** (0.01 mol) was added. The reaction mixture was refluxed for 2hr for each and then left overnight at RT. After addition of water (50 mL), the precipitate that formed was

Table VI — Acute toxicity (ALD_{50}) of the synthesized compounds (mg/kg)

Compd	ALD_{50} (mg/kg)
3a	1211.39±0.13
3b	1432.61±0.11
4a	1564.76±0.18
4b	1543.87±0.11
6a	1100.98±0.19
6b	1456.55±0.14
7a	1245.56±0.16
7b	1456.55±0.14
8a	1231.45±0.13
8b	1271.99±0.13
9a	1231.45±0.13
9b	1271.99±0.13
13a	1614.12±0.14
13b	1112.1±0.12
14a	1234.33±0.18
14b	1222.47±0.13

Table VII — The anti-inflammatory and prostaglandin E_2 (PGE_2) inhibitory activities of the synthesized compounds using carrageanan-induced paw edema in rats

Compd	Dose mg/ kg	% Protection against edema	% inhibition of plasma (PGE_2)
3a	2.5	49.17	38.99
	5	56.90	45.16
3b	2.5	46.17	34.16
	5	54.18	43.46
4a	2.5	76.77	68.16
	5	77.80	76.88
4b	2.5	77.16	76.11
	5	79.98	78.89
6a	2.5	66.17	57.16
	5	68.15	59.11
6b	2.5	68.16	59.16
	5	72.15	61.11
7a	2.5	75.26	64.18
	5	78.88	77.98
7b	2.5	76.16	67.21
	5	79.66	79.66
8a	2.5	50.16	40.16
	5	64.29	52.13
8b	2.5	48.99	36.19
	5	57.14	45.85
9a	2.5	50.16	40.16
	5	64.29	52.13
9b	2.5	48.99	36.19
	5	57.14	45.85
13a	2.5	70.77	60.61
	5	72.67	61.34
13b	2.5	71.18	60.44
	5	74.66	64.00
14a	2.5	61.17	51.17
	5	72.10	61.19
14b	2.5	68.12	46.50
	5	79.83	60.01
Indomethacin	2.5	47.51	65.00
	5	51.00	75.14

filtered off, washed with water, air dried and purified by recrystallization from aqueous ethanol.

Preparation of 4-methoxy (9a) and 4,9-dimethoxy (9b) -5-oxo-6-(1-acetyl-3-oxo-4-cyano-5-methyl-pyrazolidin-5-yl)furo(3,2-g) benzopyranes

Compounds **9a,b** were prepared through the reaction of compound **1c** or **1d** with **B** using the above mentioned procedure with reflux time ~ 7 hr.

Preparation of 4-methoxy (13a) and 4,9-dimethoxy (13b)- 6-hydroxy-5-(3-oxo-4-cyano-5-methyl-pyrazolidin-5-yl) benzofurans

A mixture of compound **10a** or **10b** (0.01 mol) and 2-cyanoacetohydrazide **A** (0.99 g, 0.01 mol) in acetic acid (95%, 10 mL) was refluxed for 3 hr. The solid that formed after evaporation of the solvent washed with water, air dried and purified by recrystallization from diethyl ether.

Preparation of 4-methoxy (14a) and 4,9-dimethoxy (14b)-6-hydroxy-5-(1-acetyl-3-oxo-4-cyano-5-methyl-pyrazolidin-5- yl) benzofurans

To a solution of 2'-acetyl-2-cyanoacetohydrazide **B** (1.41 g, 0.01 mol) in dioxane (15 mL) was added compound **10a** or **10b** (0.01 mol). The reaction mixture was refluxed for 11 hr. The reaction mixture was poured into water and the solid product so formed was filtered off, washed with water, air dried and purified by recrystallization from aqueous ethanol.

Conclusion

From the obtained data (**Tables VI** and **VII**) it is obvious that, all the tested compounds showed potent anti-inflammatory activity with potent prostaglandin inhibition at the two dose levels (2.5 and 5 mg) tested. All the tested compounds are more potent than Indomethacin in the percentage of protection against edema whereas, only **4a,b** and **7a,b** are more potent than Indomethacin in the percentage of plasma PGE_2 at the two levels, this may be due to the presence of bis-pyrazole moiety in **4a,b** and bis-pyrazolidine in **7a,b**.

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