Synthesis and COX inhibition of 7-R$_1$-8-R$_2$-1-ethyl-3,4-dimethyl-4,10-dihydro-1*H*-pyrazolo[3,4-*c*][1,5]benzodiazocine-5,11-diones

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Abstract

The title compounds were easily synthesized by reacting the 4-aminopyrazole hydrochloride **2** and the substituted 2-nitrobenzoyl chlorides **3a-d**. The obtained 2-nitrobenzamides **4a-d** were methylated and then reduced to give the corresponding amines **6a-d**. These were hydrolyzed then directly converted into 4,10-dihydro-1*H*-pyrazolo[3,4-*c*][1,5]benzodiazocine-5,11-diones **1a-d** by the action of SOCl$_2$ in benzene. These were tested for their COX inhibitory activity, showing an inhibitory profile against both COX-1 and COX-2, being slightly more selective against COX-2 with a percentage of inhibition, at the concentration of 10 µM, in the range 42.0 – 55.0.

Keywords: Pyrazole, [1,5]benzodiazocine, 4,10-dihydro-1*H*-pyrazolo[3,4-*c*][1,5]benzodiazocin-5,11-diones, COX inhibitors

Introduction

The main part of our research has been devoted to synthetic methods leading to new ring systems, containing the pyrazole nucleus, as a pharmacophore moiety for potential drugs. In particular, the pyrazole nucleus represents a very attractive scaffold to obtain new molecules endowed with anti-inflammatory activity. As an example, the central ring of Celecoxib, one of the most COX-2- selective inhibitors, has a pyrazole nucleus.[1] Moreover, several Celecoxib analogs showing interesting selective COX-2 inhibition have been synthesized.[2-5] Finally, selective COX-2- inhibiting activity for 1*H*-pyrazolylbenzo[*d*]thiazoles, 1*H*-pyrazolylbenzo[*d*]oxazoles, and 1*H*-pyrazolylbenzo[*d*]imidazoles, as well as bicyclic pyrazoles have been described.[6,7]

Recently, in the course of our studies on pyrazolodiazocines, we have reported the successful procedure affording novel pyrazolo[3,4-*c*][1,6]benzodiazocin-11-ones[8] and pyrazolo[3,4-*c*][1,5]benzodiazocine-5-ones.[9]

As an extension of our research, in order to gain more insight into the pyrazolobenzodiazocine series, and particularly with regard to their analgesic and/or anti-inflammatory activity, we sought to synthesize novel pyrazolo[3,4-*c*][1,5]benzodiazocine -5,11-dione derivatives of type **1** and screen them for their ability to inhibit selectively the cyclooxygenase of type 2 (COX-2). Thus, in the present investigation pyrazolobenzodiazocines of type **1** were synthesized and evaluated for their *in vitro* COX-1/COX-2 isoenzyme inhibition.
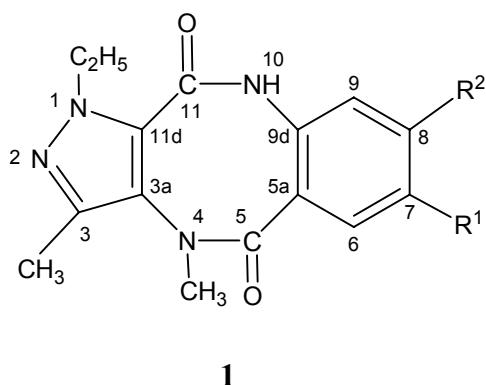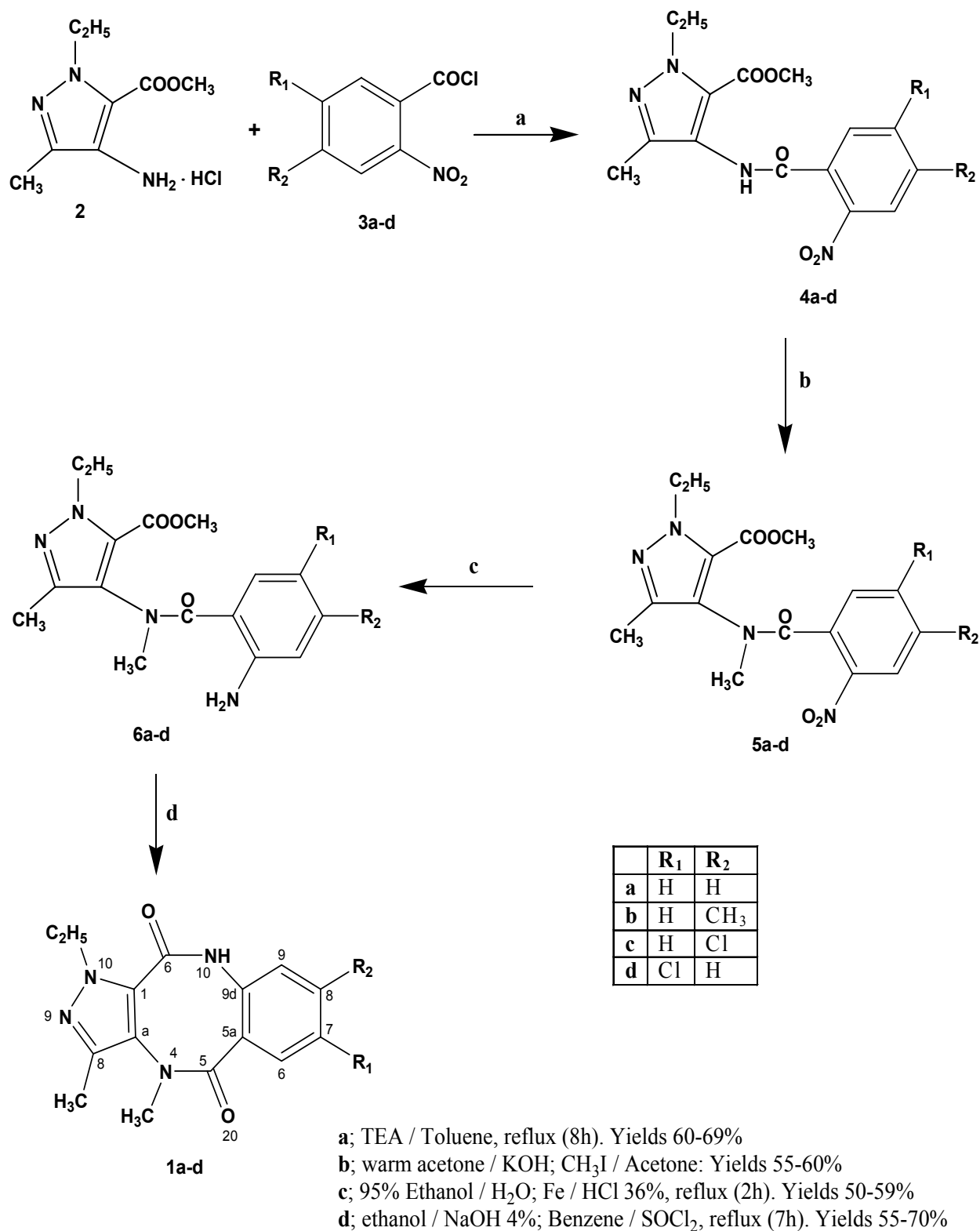

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Figure 1

We believe that the efficiency of the described synthesis of compounds **1** (see Scheme 1) could give access to a series of 4,10-dihydro-1*H*-pyrazolo[3,4-*c*][1,5]benzodiazocine-5,11-diones, for further study in order to investigate their SAR and to verify the promising results with particular regard to the "*in vivo*" anti-inflammatory activity.

Results and Discussion

Compounds **1a-d** were obtained as shown in the Scheme 1. We started our synthesis by reacting 4-aminopyrazole hydrochloride, **2**, with the substituted 2-nitrobenzoyl chlorides **3a-d** to give the corresponding 2-nitrobenzamides **4a-d** in good yields (60-69%). Reaction of these latter with methyl iodide in alkaline medium afforded the methyl derivatives **5a-d** in 53-60% yields. Reduction of these compounds with iron and hydrochloric acid in aqueous ethanol led to the corresponding amines **6a-d** in reasonable yields (53-59%).

	R$_1$	R$_2$
a	H	H
b	H	CH$_3$
c	H	Cl
d	Cl	H

a; TEA / Toluene, reflux (8h). Yields 60-69%
b; warm acetone / KOH; CH$_3$I / Acetone: Yields 55-60%
c; 95% Ethanol / H$_2$O; Fe / HCl 36%, reflux (2h). Yields 50-59%
d; ethanol / NaOH 4%; Benzene / SOCl$_2$, reflux (7h). Yields 55-70%

Scheme 1. Synthetic pathway for the formation of the compounds **1a-d**.

These products, in turn, were transformed by hydrolysis to the corresponding acids which were not isolated but converted directly into 4,10-dihydro-1*H*-pyrazolo[3,4-*c*]-[1,5]benzodiazocine-5,11-diones, **1a-d,** by the action of SOCl$_2$ in benzene solution, in 55-70 % yields. The structures of compounds **1,** and **4-6** were confirmed by spectroscopic data as well as elemental analysis. Both the ^{1}H- NMR spectra of compounds **5a-d** (in d_6- dimethyl sulfoxide, and the ^{13}C- NMR spectrum of compound **5a**, taken as an example, provided evidence for the presence of a rotational isomerism because of the hindered rotation due both to the partial double bond character of the amide C-N bond, and to the steric hindrance of the substituents in the positions 5 (ester group) and 2' (nitro group) (Tables 1 and 2).

Table 1. ^{1}H- NMR chemical shifts of compounds **5**: δ_H [ppm] (the signals related to the minor conformers are in round brackets)

Compd.	COOMe	Et	3-CH$_3$	CO-N-Sub.	NH$_2$	Ar-CH$_3$	Aryl-H
5a	3.90 (3.91)	1.09 (1.36), 4.20 (4.43)	1.98 (2.22)	3.24 (3.05)			7.37-8.29
5b	3.91 (3.95)	1.11 (1.36), 4.22 (4.45)	1.97 (2.21)	3.23 (3.04)		2.33 (2.49)	7.23-8.09
5c	3.91 (3.95)	1.13 (1.36), 4.24 (4.51)	1.98 (2.21)	3.24 (3.06)			7.39-8.06
5d	3.91 (3.94)	1.09 (1.36), 4.20 (4.44)	1.99 (2.21)	3.24 (3.07)			7.42-8.33

Table 2. ^{13}C- NMR chemical shifts of compound **5a**: δ_C [ppm] (the signals related to the minor conformer are in round brackets)

Compd.	Et	Me	Aryl-CH	Aryl-C	COOMe	N-CO	NMe
5a	15.26, 46.99 (15.49, 46.99)	10.23 (10.68)	133.94 (145.03), 130.39 (130.51), 128.80 (127.79), 123.96 (124.79)	144.93 (145.03), 143.70 (144.23), 131.61 (132.51), 127.56 (126.85), 125.19 (125.53)	158.94 (158.88), 52.39 (52.30)	166.43 (166.90)	36.21 (39.32)

In fact, the double signals for each of the carbon atoms in the ^{13}C- NMR spectrum of compound **5a** gave an account of the relative abundance of the two conformers (59-70 % for the

more abundant isomer). Thus, two signals appeared for the NCH$_3$ carbon atom, the upfield ones being more abundant. According to the literature,[10] the upfield resonance was assigned to the conformer bearing the methyl group *syn-* to the carbonyl. Also, the amide carbonyl carbon resonance of the more abundant conformer resulted in an upfield shift with respect to the corresponding isomer.

Finally, in the case of derivatives **1**, the ^{1}H-NMR spectra indicated hindered internal rotation of the N-ethyl group. In fact, the methylene protons of the ethyl group bonded at the 1- position of the tricyclic system are diastereotopic, and showed signals as multiplets in the 3.92-4.12 ppm region, due to geminal and vicinal coupling.

The ability of compounds **1a-d** to inhibit the conversion of arachidonic acid to prostaglandin H$_2$ (PGH$_2$) was determined using a COX-1/COX-2 inhibitor screening assay kit (Kit No. 560101;Cayman Chemical, Ann Arbor, MI, USA). The results reported in Table 3 show that compounds **1** are endowed with a good inhibitory activity against both COX-1 and COX-2. All compounds **1** were slightly more selective against COX-2, showing at a 10 µM concentration, a percentage of inhibition in the range 42–55. The 8-chloro derivative **1c** was the most active, with 55% of COX-2 inhibition. The most active against COX-1 was the unsubstituted derivative **1a**, with a percentage 43.1.

These preliminary results are quite encouraging and further investigation of the structure-activity and toxicity of these kind of compounds are currently underway in our group, with the aim of obtaining more COX-2- selective compounds.

Table 3. Percent inhibition of COX-1 and COX-2 at 10µM concentration of **1a-d** and 2µM for NS398 and INDO

Compound	COX-1	COX-2
1a	43.1	49.0
1b	35.2	44.2
1c	38.0	55.0
1d	24.7	42.0
NS398 [a]	39.0	69.1
INDO [b]	73.6	35.2

The determination was performed in duplicate for two independent experiments. P<0.05 Mann-Whitney test *versus* controls.
[a] NS398 = *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulphonamide. [b] INDO = Indomethacin.

Experimental Section

General Procedures. All melting points were taken on a Büchi-Tottoli capillary apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Infracord 137 spectrophotometer as Nujol mulls; ^{1}H- and ^{13}C- NMR spectra were obtained in DMSO-d_6 at 300.13 and 75.47 MHz respectively, using a Bruker AC series 300 MHz spectrometer (TMS as internal reference). Mass spectra were recorded on a JEOL jms-0I-SG-2 spectrometer at 75 eV (100μA).

General procedure for methyl 1-ethyl-3-methyl-4-(2-nitrobenzamido)-1*H*-pyrazole-5-carboxylates (4a-d)

A solution of 4-aminopyrazole hydrochloride **2** (2 mmol), the appropriate 2-nitrobenzoyl chloride derivative **3a-d** (2 mmol), and triethylamine (6 mmol) in toluene (100 mL) was heated under reflux for 8 h. The solvent was then evaporated under reduced pressure and the residue was taken up with water, filtered, and recrystallized from ethanol. **4a**: yield 65%; mp 160-161°C (white needles); IR (KBr) (cm^{-1}) 3257 (NH), 1728 and 1649 (CO); ^{1}H- NMR (δ) 1.35 (t, 3H, CH$_3$), 2.19 (s, 3H, 3-CH$_3$), 3.85 (s, 3H, COOCH$_3$), 4.42 (q, 2H, CH$_2$), 7.04-8.13 (m, 4H, phenyl protons), 10.13 (broad s, 1H, NH, D$_2$O -exchangeable). Anal. Calcd. for C$_{15}$H$_{16}$N$_4$O$_5$: C, 54.21; H, 4.85; N, 16.86. Found: C, 54.37; H, 4.72; N, 16.58%. **4b**: yield 69%; mp 178-179°C (white needles); IR (KBr) (cm^{-1}) 3220 (NH), 1725 and 1660 (CO); ^{1}H- NMR (δ) 1.33 (t, 3H, CH$_3$), 2.15 (s, 3H, 3-CH$_3$), 2.48 (s, 3H, Ar-CH$_3$), 3.85 (s, 3H, COOCH$_3$), 4.39 (q, 2H, CH$_2$), 7.85-7.93 (m, 3H, phenyl), 10.05 (broad s, 1H, NH, D$_2$O-exchangeable). Anal. Calcd. For C$_{16}$H$_{18}$N$_4$O$_5$: C, 55.49; H, 5.24; N, 16.18. Found: C, 54.57; H, 5.62; N, 16.34%. **4c**: yield 61%; mp 190-191°C (white needles); IR (KBr) (cm^{-1}) 3218 (NH), 1726 and 1659 (CO); ^{1}H- NMR (δ) 1.39 (t, 3H, CH$_3$), 2.21 (s, 3H, 3-CH$_3$), 3.89 (s, 3H, COOCH$_3$), 4.46 (q, 2H, CH$_2$), 7.78-8.31 (m, 3H, phenyl), 10.24 (broad s, 1H, NH, D$_2$O-exchangeable). Anal. Calcd. For C$_{15}$H$_{15}$ClN$_4$O$_5$: C, 49.12; H, 4.12; N, 15.28. Found: C, 49.23; H, 4.35; N, 15.12%. **4d**: yield 60%; mp 193-194 °C (white needles); IR (KBr) 3270 (NH), 1732 and 1666 (CO) cm^{-1}; ^{1}H- NMR (δ) 1.33 (t, 3H, CH$_3$), 2.17 (s, 3H, 3-CH$_3$), 3.85 (s, 3H, COOCH$_3$), 4.40 (q, 2H, CH$_2$), 7.73-8.18 (m, 3H, phenyl), 10.22 (broad s, 1H, NH, deuterium oxide-exchangeable). Anal. Calcd. For C$_{15}$H$_{15}$ClN$_4$O$_5$: C, 49.12; H, 4.12; N, 15.28. Found: C, 49.33; H, 4.25; N, 15.31%.

General procedure for the synthesis of methyl 1-ethyl-3-methyl-4-(*N*-methyl-2-nitrobenzamido)-1*H*-pyrazole-5-carboxylate (5a-d)

To a solution of one of the compounds **4a-d** (3 mmol) in warm acetone (20 mL) was added powdered potassium hydroxide (10 mmol). The reaction mixture was gently refluxed while methyl iodide (40 mmol) in acetone (8 mL) was added. After 30 minutes the solution was filtered, and concentrated under reduced pressure. The precipitated product was collected and purified from ethanol. **5a**: yield 55%; mp 123-124 °C (white crystals); IR (KBr) 1723 and 1655 (CO) cm^{-1}. ^{1}H- NMR (the signals related to the minor conformer of compound **5a** are in brackets) (δ) 1.09 (1.35) (t, 3H, CH$_3$), 1.98 (2.22) (s, 3H, 3-CH$_3$), 3.24 (3.05) (s, 3H, CONCH$_3$),

3.90 (3.91) (s, 3H, COOCH$_3$), 4.20 (4.43) (q, 2H, CH$_2$), 7.37-8.29 (m, 4H, phenyl); ^{13}C- NMR (the signals related to the minor conformer of compound **5a** are in round brackets) (δ) 10.23 (10.68), 15.26 (15.49), 36.21 (39.32), 46.99 (46.99), 52.39 (52.30), 123.96 (124.79), 125.19 (125.53), 127.56 (126.85), 128.80 (127.79), 130.39 (130.51), 131.61 (132.51), 133.94 (145.03), 143.70 (144.23), 144.93 (145.03), 158.94 (158.88), 166.43 (166.90). Anal. Calcd. For C$_{16}$H$_{18}$N$_4$O$_5$: C, 55.49; H, 5.24; N, 16,18. Found: C, 55.38; H, 5.15; N, 16.38%. **5b**: yield 60%; mp 106-107°C (white crystals); IR (KBr) (cm^{-1}) 1712 and 1652 (CO); ^{1}H- NMR (δ) (the signals for the minor conformer of compound **5b** are in round brackets) 1.11 (1.36) (t, 3H, CH$_3$), 1.97 (2.21, s, 3H, 3-CH$_3$), 2.33 (2.49), s, 3H, Ar-CH$_3$, 3.23 (3.04), s, 3H, CONCH$_3$, 3.91 (3.95), s, 3H, COOCH$_3$, 4.22 (4.45), q, 2H, CH$_2$, 7.37-8.09, m, 4H, phenyl. Anal. Calcd. For C$_{17}$H$_{20}$N$_4$O$_5$: C, 56.66; H, 5.59; N, 15.55. Found: C, 56.47; H, 5.42; N, 15.44%. **5c**: yield 53%; mp 100-101°C (white crystals); IR (KBr) (cm^{-1}) 1714 and 1656 (CO); ^{1}H- NMR (δ) (the signals related to the minor conformer of compound **5c** are in round brackets) 1.13 (1.36) (t, 3H, CH$_3$), 1.98 (2.21) (s, 3H, 3-CH$_3$), 3.24 (3.06) (s, 3H, CONCH$_3$), 3.91 (3.95) (s, 3H, COOCH$_3$), 4.24 (4.51) (q, 2H, CH$_2$), 7.39-8.06 (m, 4H, phenyl protons). Anal. Calcd. for C$_{16}$H$_{17}$ClN$_4$O$_5$: C, 50.47; H, 4.50; N, 14.71. Found: C, 50.29; H, 4.55; N, 14.77%. **5d**: yield 55%; mp 80-81°C (white crystals); IR (KBr) (cm^{-1}) 1729 and 1662 (CO); ^{1}H- NMR (the signals related to the minor conformer of compound **5d** are in round brackets) (δ) 1.09 (1.36) (t, 3H, CH$_3$), 1.99 (2.21) (s, 3H, 3-CH$_3$), 3.24 (3.07) (s, 3H, CONCH$_3$), 3.91 (3.94) (s, 3H, COOCH$_3$), 4.20 (4.44) (q, 2H, CH$_2$), 7.42-8.33 (m, 4H, phenyl). Anal. Calcd. for C$_{16}$H$_{17}$ClN$_4$O$_5$: C, 50.47; H, 4.50; N, 14.71. Found: C, 50.43; H, 4.55; N, 15.42%.

General procedure for the synthesis of methyl 4-[(2-aminobenzoyl(methyl) amino]- 1-ethyl-3-methyl- 1*H*-pyrazole-5-carboxylate (6a-d)

Compounds **5a-d** (8 mmol) were dissolved in 95% ethanol (10 mL) diluted with water (3.5 mL) and cooled to 25 °C. Iron powder (2.0g, 36 mmol) was added, followed by HCl (36%, 1 mL). An exothermic reaction resulted for the next 15 minutes; after this reaction subsided, the mixture was stirred under reflux for another 2 h. The cooled reaction mixture was filtered using Celite. The filter cake was washed well with ethanol, and the filtrate was evaporated under reduced pressure. The residue was diluted with ether (100 mL), and the solution extracted with water and dilute NaOH (4%). The ether solution was dried over Na$_2$SO$_4$ and after evaporation a residue was obtained which was purified from a small amount of ethanol. **6a**: yield 50%; mp 114-115 °C (beige crystals); IR (KBr) (cm^{-1}) 3471 and 3363 (NH$_2$), 1717 and 1645 (CO); ^{1}H- NMR (δ) 1.17 (t, 3H, CH$_3$), 2.05 (s, 3H, 3-CH$_3$), 3.18 (s, 3H, CONCH$_3$), 3.86 (s, 3H, COOCH$_3$), 4.42 (q, 2H, CH$_2$), 5.41 (broad s, 2H, NH$_2$, D$_2$O-exchangeable), 6.18-6.94 (m, 4H, phenyl). Anal. Calcd. For C$_{16}$H$_{20}$N$_4$O$_3$: C, 60.75; H, 6.37; N, 17.71. Found: C, 60.66; H, 6.45; N, 17.66%.

Compound 6b. Yield 59%; mp 110-111 °C (beige crystals); IR (KBr) (cm^{-1}) 3470, 3365 (NH$_2$), 1718, 1646 (CO); ^{1}H- NMR (δ) 1.18 (t, 3H, CH$_3$), 2.01 (s, 3H, Ar-CH$_3$), 2.05 (s, 3H, 3-CH$_3$), 3.14 (s, 3H, CONCH$_3$), 3.85 (s, 3H, COOCH$_3$), 4.42 (q, 2H, CH$_2$), 5.41 (broad s, 2H, NH$_2$, D$_2$O-exchangeable), 6.02-6.53 (m, 3H, phenyl). Anal. Calcd. for C$_{17}$H$_{22}$N$_4$O$_3$: C, 61.80; H, 6.71; N,

16.96. Found: C, 61.77; H, 6.58; N, 16.74%. **6c**. Yield 53%; mp 112-113°C (white crystals); IR (KBr) (cm^{-1}) 3468 and 3360 (NH$_2$), 1718, 1647 (CO); ^{1}H- NMR (δ) 1.92 (t, 3H, CH$_3$), 2.05 (s, 3H, 3-CH$_3$), 3.17 (s, 3H, CONCH$_3$), 3.89 (s, 3H, COOCH$_3$), 4.30 (q, 2H, CH$_2$), 5.71 (broad s, 2H, NH$_2$, D$_2$O-exchangeable), 6.22-6.67 (m, 3H, phenyl). Anal. Calcd. for C$_{16}$H$_{19}$ClN$_4$O$_3$: C, 54.78; H, 5.46; N, 15.97. Found: C, 54.69; H, 5.35; N, 15.78%. **6d**: yield 59%; mp 120-121°C (white crystals); IR (KBr) (cm^{-1}) 3474 and 3372 (NH$_2$), 1716 and 1644 (CO); ^{1}H- NMR (δ) 1.17 (t, 3H, CH$_3$), 2.04 (s, 3H, 3-CH$_3$), 3.17 (s, 3H, CONCH$_3$), 3.86 (s, 3H, COOCH$_3$), 4.20-4.42 (q, 2H, CH$_2$), 5.57 (broad s, 2H, NH$_2$, D$_2$O-exchangeable), 6.57-6.97 (m, 3H, phenyl). Anal. Calcd. for C$_{16}$H$_{19}$ClN$_4$O$_3$: C, 54.78; H, 5.46; N, 15.97. Found: C, 54.89; H, 5.27; N, 15.85%.

General procedure for the synthesis of 7-R$_1$-8-R$_2$-3,4-dimethyl-1-ethyl-4,10-dihydro-1*H*-pyrazolo[3,4-*c*][1,5]benzodiazocine-5,11-diones (1a-d)

Compounds **6a-d** (3 mmol) in ethanol (30 mL) and aqueous 4% KOH solution (30 mL), were refluxed for 15 minutes and stirred at RT for 3h. The reaction mixture was cooled and acidified to pH 3.5 and the solid precipitated was collected, dissolved in benzene (30 mL), added to SOCl$_2$ (3.5 mL) and heated under reflux for 7 h. Upon evaporation the obtained product was recrystallized from ethanol. Compound **1a** was dried in a Büchi drying oven, TO-51, at reduced pressure (40 mm Hg) and 120 °C for 20 h. Product **1a**: yield 65%; mp 190-191 °C (white crystals); IR (KBr) (cm^{-1}) 3223 (NH), 1717, 1682 and 1643 (CO); MS (*m/z*) 284 (M$^+$); ^{1}H- NMR (δ) 1.20 (t, 3H, CH$_3$), 2.10 (s, 3H, CH$_3$), 3.27 (s, 3H, NCH$_3$), 3.92-4.12 (q, 2H, CH$_2$), 7.10-7.39 (m, 4H, phenyl), 10.30 (broad s, 1H, NH, D$_2$O-exchangeable); ^{13}C- NMR (δ) 10.97, 15.46, 35.59, 44.95, 123.69, 126.10, 127.51, 128.22, 130.02, 130.21, 134.31, 134.71, 149.97, 161.78, 168.10. Anal. Calcd. for C$_{15}$H$_{16}$N$_4$O$_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.46; H, 5.45; N, 19.66%. **1b**: yield 70%; mp 238-239°C (white crystals); IR (KBr) (cm^{-1}) 3167 (NH), 1717, 1670, 1654 (CO). MS (*m/z*) 298 (M$^+$); ^{1}H- NMR (δ) 1.15 (t, 3H, CH$_3$), 2.10 (s, 3H, CH$_3$), 3.23 (s, 3H, NCH$_3$), 3.24 (s, 3H, CH$_3$), 3.95-4.10 (q, 2H, CH$_2$), 6.89-7.21 (m, 3H, phenyl), 10.22 (broad s, 1H, NH, D$_2$O-exchangeable). ^{13}C- NMR (δ) 11.48, 15.95, 20.90, 36.14, 45.45, 123.73, 126.79, 128.63, 128.69, 130.25, 131.46, 134.61, 140.00, 140.94, 161.76, 168.19. Anal. Calcd. for C$_{16}$H$_{18}$N$_4$O$_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.52; H, 6.38; N, 18.74%. **1c**: yield 55%; mp 192-193°C (white crystals); IR (KBr) (cm^{-1}) 3173 (NH), 1717, 1675 and 1651 (CO); MS (*m/z*) 318 (M$^+$); ^{1}H- NMR (δ) 1.17 (t, 3H, CH$_3$), 2.10 (s, 3H, CH$_3$), 3.27 (s, 3H, NCH$_3$), 3.96-4.07 (q, 2H, CH$_2$), 7.22-7.38 (m, 3H, phenyl protons), 10.36 (broad s, 1H, NH, D$_2$O-exchangeable). ^{13}C- NMR (δ) 10.97, 11.44, 35.67, 45.07, 123.50, 125.85, 127.72, 129.84, 129.97, 133.19, 133.93, 136.16, 141.12, 161.53, 167.11. Anal. Calcd. for C$_{15}$H$_{15}$ClN$_4$O$_2$: C, 56.52; H, 4.74; N, 17.58. Found: C, 56.77; H, 4.65; N, 17.78%.

Compound 1d. Yield 58%; mp210-211 °C (white crystals); IR (KBr) (cm^{-1}) 3186 (NH), 1717, 1676 and 1655 (CO); MS (m/z) 318 (M$^+$); ^{1}H- NMR (δ) 1.36 (t, 3H, CH$_3$), 2.12 (s, 3H, CH$_3$), 3.25 (s, 3H, NCH$_3$), 3.95-4.12 (q, 2H, CH$_2$), 7.13-7.45 (m, 3H, phenyl protons), 10.36 (broad s, 1H, NH, D$_2$O-exchangeable). ^{13}C- NMR (δ) 10.97, 15.47, 35.62, 45.01, 123.43, 127.87, 128.03,

129.94, 131.72, 133.73, 135.93, 141.19, 161.59, 166.54. Anal. Calcd. for $C_{15}H_{15}ClN_4O_2$: C, 56.52; H, 4.74; N, 17.58. Found: C, 56.59; H, 4.79; N, 17.65%.

Biology

***In vitro* cyclooxygenase inhibition assay.** The reference compounds (indomethacin and NS398) were purchased from Cayman Chemical, Ann Arbor, MI (cat. N. 70270 and 70590 respectively). They are used at the final concentration of 0.2μM, according to the manufacturer's instructions. Compounds **1a-d** were tested for their ability to inhibit COX-1 and COX-2 using a COX-(ovine) inhibitor screening kit (Catalogue No 560101, Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid (AA) to PGH_2 $PGF_{2\alpha}$ produced from PGH_2 by reduction with stannous chloride is measured by enzyme immunoassay (ACE competitive EIA). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (950μL, 0.1 M Tris-HCl pH 8.0 containing 5 mM EDTA and *2* mM phenol) with either COX 1 or COX-2 (10μL) enzyme in the presence of heme (10 μL) was added 20 μL of 10μM concentration of test drug solutions. These solution were incubated for a period of 2 min at 37 °C after which 10 μL of AA (100 μM) was added, and the COX reaction was stopped by the addition of 50 μL of 1 M HCl after 2 min. PGF_2 produced from PGH_2 by reduction with stannous chloride, was measured by enzyme immunoassay.

This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells, since the concentration of the PG tracer is held constant while the concentration of PGs varies. This antibody-PG complex binds to a mouse anti-rabbit monoclonal antibody that had been attached to the well previously. The plate is washed to remove any unbound reagents and then Ellman's Reagent, which contains the substrate to acetylcholinesterase is added to the well. The product of this emymatic reaction produces a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation absorbance $\propto$ [bound PG tracer] $\propto$ 1/PGs. Percent inhibition was calculated by comparison of compound-treated to various control incubations (duplicate determinations).

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