Synthesis and Analgesic Activity of 2-Methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetic Acid Methyl Esters, Acetic Acids, and Acetamides

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Key Words: *1,4-Dihydropyridines; acetic acid esters; acetic acids; acetamides, analgesic activity*

Summary

A group of 2-methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetic acid methyl esters (**7**), weak acetic acids (**8**), and acetamides (**9**) were designed for evaluation as less acidic non-ulcerogenic non-steroidal antiinflammatory drugs (NSAIDs). In this respect, the model compound 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetic acid (**8a**), unlike traditional arylacetic acid NSAIDs, was shown to be a weak acid with a pK_a of 9.17. In contrast to arylacetic acid NSAIDs, the α-methylacetic acid sodium salt of **8a**, or the methyl α-methylacetate ester (**7a**) did not inhibit cyclooxygenase-1 (COX-1) or -2 (COX-2). *In vitro* stability studies showed that the methyl α-methylacetate ester (**7a**) acts as a prodrug to the α-methylacetic acid derivative (**8a**), undergoing rapid (< 10 minutes) and quantitative conversion upon incubation with rat plasma, or incubation with rat liver homogenate ($t_{1/2}$ = 25 min). In contrast, the α-methylacetamide (**9a**) underwent negligible (< 2%) conversion to the α-methylacetic acid derivative (**8a**) upon incubation with either rat plasma, or rat liver homogenate, for incubation times up to 24 h. The effect of a C-3 *para*-substituted-benzoyl substituent (R^1 = H, Cl, Me), a C-4 substituent (R^2 = aryl, benzyl, cyclohexyl, alkyl), and the nature of the N^1-acetic acid moiety [methyl ester (R^3 = OMe), acetic acid (R^3 = OH), acetamide ($R^3 = NH_2$)] on analgesic activity was determined using the 4% NaCl-induced abdominal constriction (writhing) assay. Compounds **7–9** inhibited writhing 27–95% relative to the reference drug aspirin (58% inhibition). The analgesic potency with respect to the *para*-benzoyl substituent was H > Cl or Me. Although the effect of the C-4 R^2-substituent on analgesic activity was variable within the ester, acid and amide sub-groups of compounds, compounds having a R^2-cyclohexyl substituent generally provided superior analgesic activity relative to those having a lipophilic alkyl substituent. The nature of the R^3-substituent (OMe, OH, NH_2) was a determinant of analgesic activity where the potency order was acetic acid methyl ester > acetic acid or acetamide, except when the C-4 R^2-substituent was cyclohexyl or benzyl where the potency order was acetamide > acetic acid methyl ester or acetic acid. Reduction of the 5,6-olefinic bond of the 1,4-dihydropyridyl compound (**9a**, 94% inhibition) to the corresponding 1,2,3,4-tetrahydropyidyl derivative (**10**, 69% inhibition) reduced analgesic activity.

Introduction

In an earlier study, we described the synthesis and characterization of 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetic acid methyl ester (**1a**), acetic acid sodium salt (**1b**), and acetamide (**1c**) diastereomers (4*R**,2*R**; 4*S**,2*S**; 4*R**,2*S**; 4*S**,2*R**)[1]. Aryl- and heteroarylacetic acid derivatives that exhibit analgesic and non-steroidal anti-inflammatory (NSAID) activities possess several common structural features. These include, (a) a carboxyl group or its equivalent (ester, amide) separated by one carbon atom from a flat aromatic nucleus, and (b) one or more large lipophilic groups attached to the aromatic nucleus that is two, three, or four carbon or heteratoms removed from the point of attachment of the acetic acid side chain[2]. The observation that the acetic acid derivatives (**1**) bear some structural similarity to the NSAID ketoprofen (**2**)[3], and that the non-ulcerogenic antiinflammatory indan-1-acetic acid (**3**) induces minimal gastric irritation[4], prompted us to investigate the pharmacological action(s) exhibited by this novel class of compounds **1**. We now describe the synthesis and pharmacological evaluation of 2-methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetic acids esters, acetic acids and acetamides of general formula **4** wherein the R^1-substituent is H, Cl or Me, the R^2-substituent is aryl, arylalkyl, alkyl or cycloalkyl, and the R^3-substituent is OMe, OH or NH_2 (see structures in Figure 1).

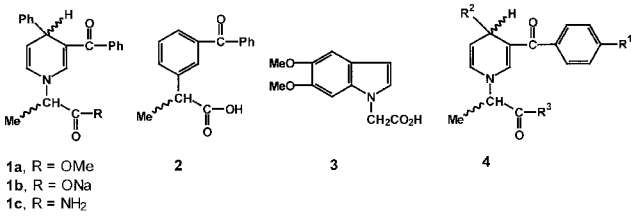

Figure 1. Structures of 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetic acid methyl ester (**1a**), acetic acid sodium salt (**1b**) and acetamide (**1c**), ketoprofen (**2**), 5,6-dimethoxyindan-1-acetic acid (**3**), and 2-methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetic acid esters (**4**, R^3 = OMe), acetic acids (**4**, R^3 = OH) and acetamides (**4**, $R^3 = NH_2$).

Chemistry

The methyl 2-methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetates (**7a–o**) were synthesized using the procedures illustrated in Scheme 1. Thus, reaction of the 3-benzoylpyridine compound (**5**, R^1 = H, Cl, Me) with racemic methyl 2-bromopropionate in acetone at reflux temperature afforded the corresponding pyridinium bromide salt (**6**). The subsequent regiospecific copper-catalyzed reaction[5] of a Grignard reagent (R^2MgCl; R^2 = Ph, 4-Cl-C_6H_4-, 4-Me-C_6H_4-, cyclohexyl, $PhCH_2$, *n*-Bu, *i*-Bu, *n*-hexyl,

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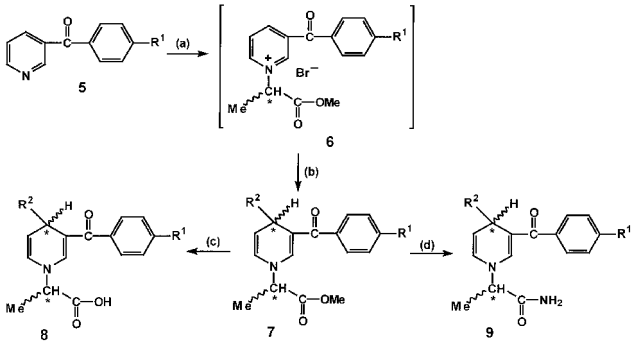
n-tetradecyl) with the pyridinium salt (**6**) yielded the corresponding methyl acetate ester (**7a–o**) in 14–91% yields. No product arising from reaction of the benzoyl substituent of the pyridinium salt (**6**) with the Grignard reagent was observed. This is attributed to the fact that pyridinium salts are so reactive to Grignard reagents in the presence of cuprous iodide that selective reaction at C-4 of the pyridinium salt takes place even when a reactive substituent, such as a ketone, is present at C-3[6]. The presence of an electron-withdrawing 3-benzoyl group, that is conjugated with the enamine moiety, stabilizes the 1,4-dihydropyridyl ring system which prevents aromatization[7].

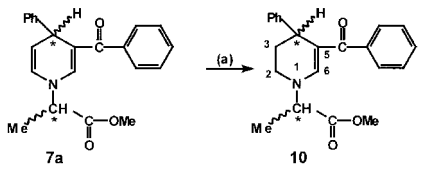


Scheme 1. Reagents and conditions: (a) racemic $BrCH(Me)CO_2Me$, acetone, reflux, 8 h; (b) 0.1 equiv. CuI, THF, R^2MgCl, –23 °C, 0.5 h; (c) aqueous NaOH, EtOH-H_2O, 25 °C, 2 h; (d) NH_3, MeOH, 25 °C, 48 h.

The methyl acetate esters (**7a–o**), which contain two asymmetric carbons at the dihydropyridyl C-4 and α-methyl acetate *methine*, positions exist as a mixture of four diastereomers (*SS*, *SR*, *RS*, *RR*). The ^{1}H NMR spectra of the methyl acetate derivatives (**7a–o**) exhibit dual resonances (approximate ratio of about 1:1) for some of the dihydropyridyl ring and ester O*Me* protons, as reported previously[1].

Hydrolysis of the methyl acetates (**7**, mixture of four diastereomers) with aqueous sodium hydroxide at 25 °C for two hours afforded the corresponding acetic acid analogs (**8a–g**, mixture of four diastereomers) in 21–78% yields. Ammonolysis of the methyl acetate esters (**7**, mixture of four diastereomers) yielded the corresponding acetamide analogs (**9a–g**, mixture of four diastereomers) in 48–95% yields. A complete list of the compounds prepared (**7–9**) is provided in Table 1.

Hydrogenation of methyl 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetate (**7a**, mixture of four diastereomers) using 10% Pd/C and hydrogen gas afforded methyl 2-methyl-2-[1-(4-phenyl-5-benzoyl-1,2,3,4-tetrahydropyridyl)]acetate (**10**, 47%, mixture of four diastereomers) as illustrated in Scheme 2.



Scheme 2. Reagents and conditions: (a) H_2 gas at 30 psi, 10% Pd/C, EtOAc,

Results and Discussion

The 1,4-dihydropyridyl ring system present in 2-methyl-2-[1-(3-benzoyl-4-aryl (cycloalkyl, arylalkyl or alkyl)-1,4-dihydropyridyl)]acetic acid analogs (**7–9**) possesses conformational and steric properties that are significantly different from those present in traditional benzoylarylacetic acids (NSAIDs) such as ketoprofen (**2**). Accordingly, the 1,4-dihydropyridyl ring system is more puckered than the planar aryl(heteroaryl) ring system due to distortion at the 1,4-dihydropyridyl N-1 and C-4 positions (see Figure 2). Theoretical ab initio STO-3G calculations for 4-phenyl-3,5-dicarboxy-1,4-dihydropyridine showed that a 1,4-dihydropyridine boat-conformation is more favored relative to a planar ring conformation, that ring distortion is greater at C-4 than at N-1, and that a pseudo-axial orientation of the C-4 phenyl moiety is unequivocally favored[8,9]. These conformational differences, together with steric and physicochemical effects due to the 1,4-dihydropyridyl N-1, C-3 and C-4 substituents, are expected to change the overall size of the molecule, tissue biodistribution and potential mechanism of action of compounds **7–9**.

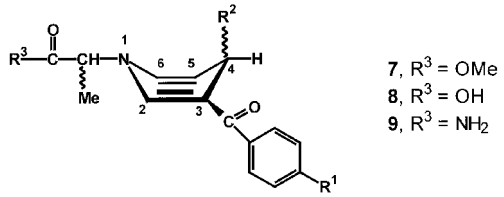
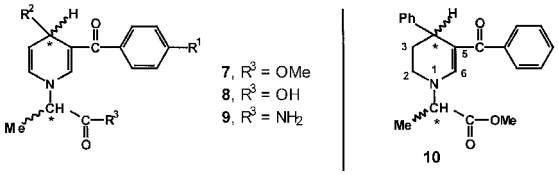


Figure 2. Boat-shaped structure for 2-methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetic acid methyl esters (**7**), acetic acids (**8**) and acetamides (**9**).

There are two contributing factors responsible for the gastrointestinal ulcerogenicity and hemorrhage resulting from NSAID therapy. These include local irritation of the gastric mucosa due to drug acidity, and inhibition of Prostaglandin E_2 (PGE_2) biosynthesis in the gastric mucosal barrier[11]. Attachment of the α-methylacetic acid moiety to the 1,4-dihydropyridyl N-1 sp^3 hybridized nitrogen atom is expected to reduce acidity significantly since the N-substituted-α-methylacetic acid moiety (**8**) may be viewed as an alanine [$NCH(Me)CO_2H$] moiety. It was therefore expected that α-methylacetic acids (**8**) would be weak acids like alanine (pK_a = 9.87) or phenylalanine (pK_a = 9.24), compared to stronger acids such as acetic acid (pK_a = 4.74) or phenylacetic acid (pK_a = 4.25). The pK_a of 9.17 for 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetic acid (**8a**), determined using a potentiometric method {Henderson-Hasselbach equation; pK_a = pH + log [HA] / log [A^-]} reported by Albert and Searjent[10], was in good agreement with the postulated pK_a value. The weak α-methylacetic acids (**8**) would therefore be expected to cause less local gastric irritation than commonly used acidic NSAIDs, since many acidic compounds have a tendency to accumulate in the stomach wall soon after oral administration [12]. In contrast, non-acidic compounds generally have less tendency to accumulate in stomach tissue and show better gastrointestinal tolerance [13]. The ulcerogenic liability of **8a** was determined using a modified method based on the procedure of Nagai *et*

Table 1. Physical data and analgesic activities for methyl 2-methyl-2-[1-(3-*para*-substituted-benzoyl-4-substituted-1,4-dihydropyridyl)]acetates (**7a–o**), 2-methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetic acids (**8a–g**), 2-methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetamides (**9a–g**), and methyl 2-methyl-2-[1-(4-phenyl-5-benzoyl-1,2,3,4-tetrahydropyridyl)]acetate (**10**).



Cmpd	R^1	R^2	R^3	Mp °C	Yield %	Exact Mass Calcd.	Exact Mass Found	Analgesic activity % inhibition[a]
7a	H	Ph	OMe	–	69[b]	–	–	94.5 ± 1.5
7b	H	4-Cl-C_6H_4-	OMe	oil	51	381.1106	381.1104	57.5 ± 2.0
7c	H	4-Me-C_6H_4-	OMe	oil	28	361.1678	361.1685	65.4 ± 2.1
7d	H	cyclohexyl	OMe	oil	91	353.1991	270.1129[c]	62.8 ± 1.8
7e	H	$PhCH_2$	OMe	oil	28	361.1678	361.1678	57.5 ± 3.7
7f	H	Me	OMe	oil	21	–	ND[d]	61.1 ± 16.4
7g	H	*n*-Bu	OMe	oil	23	327.1834	326.1716[e]	66.5 ± 1.4
7h	H	*i*-Bu	OMe	oil	14	327.1834	327.1833	72.8 ± 3.1
7i	H	$Me(CH_2)_5$	OMe	oil	72	–	ND[f]	72.2 ± 7.1
7j	H	$Me(CH_2)_{13}$	OMe	oil	80	–	ND[g]	39.7 ± 2.6
7k	Cl	Ph	OMe	oil	22	381.1132	381.1131	80.0 ± 2.5
7l	Cl	4-Cl-C_6H_4-	OMe	oil	27	415.0739	415.0731	47.6 ± 3.5
7m	Cl	$PhCH_2$	OMe	oil	21	395.1288	304.0744[h]	53.0 ± 2.6
7n	Cl	cyclohexyl	OMe	oil	39	387.1601	304.0739[i]	51.0 ± 5.3
7o	Me	Ph	OMe	oil	50	361.1678	361.1674	62.7 ± 1.7
8a	H	Ph	OH	93–95	30	–	ND[j]	30.1 ± 4.7
8b	H	4-Cl-C_6H_4-	OH	98–100	52	–	ND[j]	43.4 ± 2.1
8c	H	cyclohexyl	OH	84–86	54	–	ND[j]	62.8 ± 1.8
8d	H	$PhCH_2$	OH	83–85	21	–	ND[j]	NT[k]
8e	H	Me	ONa	105–115	65	–	ND[l]	30.6 ± 2.3
8f	H	*n*-Bu	OH	68–70	37	–	ND[j]	NT
8g	H	*i*-Bu	OH	89–90	78	318.1368	318.1362	NT
9a	H	Ph	NH_2	–	67[b]	–	–	75.9 ± 2.3
9b	H	4-Me-C_6H_4-	NH_2	95–96	62	346.1681	346.1678	62.5 ± 5.7
9c	H	cyclohexyl	NH_2	90–92	95	338.1994	255.1126[m]	95.6 ± 3.6
9d	H	$PhCH_2$	NH_2	85–87	73	344.1525	344.1521	75.5 ± 2.9
9e	H	*n*-Bu	NH_2	70–73	60	312.1837	312.1837	NT
9f	H	*i*-Bu	NH_2	78–80	48	312.1837	312.1837	NT
9g	H	$Me(CH_2)_{13}$	NH_2	121–123	46	–	ND[n]	27.6 ± 11.3
10				oil	47	–	ND[o]	68.6 ± 5.0
Aspirin								57.8 ± 2.8

[a] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction (writhing) assay. The result is the mean value ± SEM using five animals following a 50 mg/kg intraperitoneal dose of the test compound.
[b] The synthesis and characterization was reported previously[1].
[c] 270.1129 $[M - cyclohexyl]^+$
[d] ND = not determined. Anal. calcd. for $C_{17}H_{19}NO_3$: C 71.54, H 6.72, N 4.91. Found: C 71.39, H 6.66, N 4.93.
[e] 326.1716 $[M - 1]^+$
[f] Anal. calcd. for $C_{22}H_{29}NO_3$: C 74.33, H 8.22, N 3.94. Found: C 74.22, H 8.38, N 4.14.
[g] Characterized as the acetamide derivative **9g**.
[h] 304.0744 $[M - benzyl]^+$
[i] 304.0739 $[M - cyclohexyl]^+$
[j] The compound was characterized as the methyl ester (**7**) prepared by adding a solution of diazomethane in MeOH to a solution of the acid (**8**). The ^1H NMR of the methyl ester prepared in this way was identical to the corresponding methyl ester (**7**).
[k] NT = not tested.
[l] Anal. calcd. for $C_{16}H_{16}NO_3Na.1/2H_2O$: C 63.57, H 5.67, N 4.63. Found: C 63.97, H 5.36, N 4.34.
[m] 255.1126 $[M - cyclohexyl]^+$; Anal. calcd. for $C_{21}H_{26}N_2O_2.1/4H_2O$: C 73.55, H 7.79, N 8.17. Found: C 73.96, H 7.71, N 8.07.
[n] Anal. calcd. for $C_{29}H_{44}N_2O_2$: C 76.95, H 9.80, N 6.19. Found: C 76.82, H 9.84, N 6.22.
[o] Anal. calcd. for $C_{23}H_{23}NO_2.1/2H_2O$: C 73.65, H 6.42, N 3.91. Found: C 73.30, H 6.36, N 3.94.

al. [14]. Although Ibuprofen was ulcerogenic in rats (UD_{50} = 250 mg/kg po), **8a** was completely devoid of any ulcerogenic effect for a single 1200 mg/kg po dose at eight hours post drug administration. A subsequent chronic ulcerogenicity study showed that **8a**, administered at 600 mg/kg po twice a day for six days, was completely devoid of any gastric irritation or ulcerogenicity.

The methyl α-methyl acetates (**7**), and α-methyl acetamides (**9**), could serve as prodrugs to α-methyl acetic acids (**8**) upon bioconversion by esterase(s), and amidase(s), respectively. The *in vitro* stability of methyl 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetate (**7a**) and the corresponding acetamide derivative (**9a**) were therefore investigated. Incubation of **7a** with rat plasma at 37 °C resulted in its rapid, and quantitative, conversion to the α-methyl acetic acid (**8a**) in less than 10 min. A similar incubation of **7a** with rat liver homogenate resulted in a slower rate of ester cleavage ($t_{1/2}$ = 25 min). In contrast, the acetamide (**9a**) underwent negligible (< 2%) conversion to **8a** upon incubation with either rat plasma, or rat liver homogenate, for incubation times up to 24 h.

The ability of the α-methylacetic acid derivative (**1b**, sodium salt of **8a**) and the methyl α-methylacetate (**7a**) to inhibit cyclooxygenase-1 (COX-1) and -2 (COX-2) was investigated in view of their structural similarity to NSAIDs such as ketoprofen (**2**). These studies showed that neither **1b** or **8a** inhibited unstimulated COX-1 activity as measured by the production of thromboxane B_2 (TXB_2), or COX-2 activity as measured by lipopolysaccaride stimulated PGE_2 production[15], at a 100 mg/kg oral dose in rats. Thus, this class of α-methylacetic acid derivatives (**7–8**), unlike traditional NSAIDs, do not act as inhibitors of the COX-1 and COX-2 isozymes.

The acetic acid methyl esters (**7**), acetic acids (**8**) and acetamides (**9**) were investigated to determine the effect of a *para*-benzoyl substitutent (R^1 = H. Cl, Me), the C-4 substituent (R^2 = phenyl, benzyl, cyclohexyl, alkyl), and the nature of the R^3-substituent (OMe, OH, NH_2) upon analgesic activity. Analgesic activity was determined using the 4% NaCl-induced writhing assay and the results are summarized in Table 1. Compounds **7–9** inhibited writhing 27–95% relative to the reference drug aspirin (58% inhibition) for a 50 mg/kg intraperitoneal dose. In the acetic acid methyl ester series (**7**), the relative potency order with respect to the R^1-*para*-benzoyl substituent was H > Cl and H > Me [H (**7a**) > Cl (**7k**); H (**7b**) > Cl (**7l**); H (**7e**) ≥ Cl (**7m**); H (**7d**) > Cl (**7n**); H (**7a**) > Me (**7o**)] The effect of the C-4 R^2-substituent on analgesic activity was variable within the acetic acid methyl ester [Ph (**7a**) > *i*-Bu (**7h**) = $Me(CH_2)_5$ (**7i**) > *n*-Bu (**7g**) > 4-Me-C_6H_4- (**7c**) ≈ cyclohexyl (**7d**) = Me (**7f**) benzyl (**7e**) and 4-Cl-C_6H_4- (**7b**) > $Me(CH_2)_{13}$ (**7j**)], acetic acid [cyclohexyl (**8c**) > 4-Cl-C_6H_4- (**8b**) > Ph (**8a**) = Me (**8e**)], and acetamide [cyclohexyl (**9c**) > Ph (**9a**) = benzyl (**9d**) > 4-Me-C_6H_4- (**9b**) > $Me(CH_2)_{13}$ (**9g**)] series of compounds. In general, the C-4 R^2-cyclohexyl compounds show superior analgesic activity, whereas those possessing a more lipophilic R^2 = $Me(CH_2)_{13}$ substituent were the least active analgesic agents. Overall the nature of the R^3-substituent (OMe, OH, NH_2) was a determinant of activity where the potency order profile was acetic acid methyl ester [**7**, R^2 = Ph, 4-Cl-C_6H_4-, 4-Me-C_6H_4-, Me, $Me(CH_2)_{13}$] > acetic acid (**8**) or acetamide (**9**), except when the C-4 R^2-subsituent was cyclohexyl or benzyl where the potency order was acetamide > acetic acid methyl ester or acetic acid. These comparative data suggest there may be a cooperative effect between the R^2- and R^3-substituents with respect to analgesic activity. Reduction of the 5,6-olefinic bond of the 1,4-dihydropyridyl compound (**9a**, 94% inhibition) to the corresponding 1,2,3,4-tetrahydropyridyl derivative (**10**, 69% inhibition) reduced analgesic activity. Methyl 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]ac etate (**7a**) and 2-methyl-2-[1-(3-benzoyl-4-cyclohexyl-1,4-dihydropyridyl)]acetamide (**9c**) exhibited the most potent analgesic activity (95% inhibition) relative to the reference drug aspirin (58% inhibition) for a 50 mg/kg intraperitoneal dose.

Acknowledgments

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Experimental

Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer. Chemical shifts are reported in ppm (δ) values relative to TMS as internal reference. The assignment of exchangeable protons (O*H*, N*H*$_2$) was confirmed by the addition of $[D_2]H_2O$. Infrared spectra were acquired using a Nicolet 5DX-FT spectrometer. Exact Mass measurements (HRMS) were obtained using an AEI MS-50 mass spectrometer (electron impact). Measurements of pH were performed using an Orion Model SA520 digital pH meter. Column chromatography was performed using Merck 7734 (100–200 mesh) silica gel. Preparative thin-layer chromatography (PTLC) was carried out using Camag Kieselgel DF-5 plates, 1 mm in thickness, activated at 120 °C overnight prior to use. Tetrahydrofuran (THF) was dried over sodium-benzophenone and distilled just prior to use. All chemical reagents, organometallic reagents in "sure-sealed" containers, were purchased from the Aldrich Chemical Co. Lipopolysaccaride from *E. coli* and indomethacin were obtained from the Sigma Chemical Co.

*General Method for the Preparation of Methyl 2-methyl-2-[1-(3-para-substituted-benzoyl-4-substituted-1,4-dihydropyridyl)]acetates **7a–o***

A solution of the respective 3-benzoylpyridine (**5**, 10.9 mmol) and *rac*-methyl 2-bromopropionate (2.6 g, 16.4 mmol) in dry acetone (20 ml) was heated at reflux for 8 h. After cooling to 25 °C, the solid was filtered and dried to give the corresponding pyridinium bromide salt (**6**, 70–75% yield), which was used immediately in the subsequent reaction. A mixture of **6** (4.3 mmol) in dry THF (50 ml) was stirred under a nitrogen atmosphere until the solution became homogeneous. The reaction mixture was cooled to –23 °C in a dry ice-CCl_4 bath, a solution of the Grignard reagent R^2MgCl (R^2 = Ph, 4-Cl-C_6H_4-, $PhCH_2$, 4-Me-C_6H_4- cyclohexyl, Me, *n*-Bu, *i*-Bu, $Me(CH_2)_5$, $Me(CH_2)_{13}$; 6.4 mmol of a 2 M solution in THF) was added dropwise, and the reaction mixture was maintained at –23 °C for 30 min prior to warming to 25 °C. The reaction mixture was stirred for a further 1.5 h before quenching by addition of saturated aqueous NH_4Cl (5 ml). Diethyl ether (30 ml) was added, the organic layer was separated and washed successively with solutions of 30% NH_4OH:saturated NH_4Cl (3:1, *v/v*; 30 ml), water (2 × 10 ml), and then brine (10 ml). The organic layer was dried ($MgSO_4$), and the solvent was removed *in vacuo* to yield a brown oil. This oil was purified by elution from a silica gel column using an EtOAc-hexane gradient going from 5:95 to 15:85 v/v as eluent to afford the respective product **7a–o** as an oil (mixture of four diastereomers) [see Table 1 for R^1-, R^2- and R^3-substituents, % yield and Exact Mass (high resolution mass spectral) data]. Infrared (IR) and ^{1}H NMR spectral data for **7a–o** are qualitatively similar except for differences with respect to the R^1- and R^2-substituents. Representative IR and ^{1}H NMR spectral data for **7b**, **7d**, **7g**, and **7k** are listed below.

Methyl 2-Methyl-2-[1-(3-benzoyl-4-para-chlorophenyl-1,4-dihydropyridyl)]acetate ***7b***

IR (film): ν = 1745 cm^{-1} (CO_2), 1679 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 1.44 (d, $J_{Me,CH}$ = 7.5 Hz, 3H, CH*Me*), 3.74 [3.72] (s, 3H, O*Me*), 4.0 [4.1] (q, $J_{Me,CH}$ = 7.5 Hz, 1H, C*H*Me), 4.84 [4.82] (d, $J_{4,5}$ = 4.9 Hz, 1H, H-4), 5.06 (dd, $J_{5,6}$ = 8.2, $J_{4,5}$ = 4.9 Hz, 1H, H-5), 6.0 [6.04] (dd, $J_{5,6}$ = 8.2, $J_{2,6}$ = 1.5 Hz, 1H, H-6), 6.98 [6.96] (d, $J_{2,6}$ = 1.5 Hz, 1H, H-2), 7.20–7.50 (m, 9H, phenyl hydrogens). The ratio of the major:minor set of resonances was about 3:2 with the δ values for the minor set of resonances when present shown in square brackets.

Methyl 2-Methyl-2-[1-(3-benzoyl-4-cyclohexyl-1,4-dihydropyridyl)]acetate ***7d***

IR (film): ν = 1753 cm^{-1} (CO_2), 1671 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 1.0–1.3 (m, 6H, cyclohexyl H-3, H-4, H-5), 1.46 [1.44] (d, $J_{Me,CH}$ = 7.4 Hz, 3H, CH*Me*), 1.60–1.82 (m, 5H, cyclohexyl H-1, H-2, H-6), 3.66–3.74 (m, 1H, H-4), 3.76 [3.75] (s, 3H, O*Me*), 3.92 (q, $J_{CH,Me}$ = 7.4 Hz, 1H, C*H*Me), 4.92–5.04 (m, 1H, H-5), 6.02 [6.00] (d, $J_{5,6}$ = 7.6 Hz, 1H, H-6), 6.88 (s, 1H, H-2), 7.35–7.65 (m, 5H, phenyl hydrogens). The ratio of the two sets of resonances was about 1:1 with the δ values for the second set of resonances when present shown in square brackets.

Methyl 2-Methyl-2-[1-(3-benzoyl-4-n-butyl-1,4-dihydropyridyl)]acetate ***7g***

IR (film): ν = 1745 cm^{-1} (CO_2), 1679 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 0.90 (t, $J_{CH2,Me}$ = 7.2 Hz, 1H, CH_2*Me*), 1.20–1.34 (m, 6H, *$CH_2CH_2CH_2$*CH_3), 1.38 [1.40] (d, $J_{Me,CH}$ = 6.8 Hz, 3H, CH*Me*), 3.68–3.80 (m, 1H, H-4), 3.76 [3.78] (s, 3H, O*Me*), 3.92 (q, $J_{CH,Me}$ = 6.8 Hz, 1H, C*H*Me), 4.98 (5.02] (dd, $J_{5,6}$ = 7.2, $J_{4,5}$ = 5.3 Hz, 1H, H-5), 5.94 (d, $J_{5,6}$ = 7.2 Hz, 1H, H-6), 6.86 (s, 1H, H-2), 7.30–7.56 (m, 5H, phenyl hydrogens). The ratio of the two sets of resonances was about 1:1 with the δ values for the second set of resonances when present shown in square brackets.

Methyl 2-Methyl-2-[1-(3-para-chlorobenzoyl-4-benzyl-1,4-dihydropyridyl)]acetate ***7k***

IR (film): ν = 1753 cm^{-1} (CO_2), 1679 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 1.24 (d, $J_{Me,CH}$ = 7.2 Hz, 3H, CH*Me*), 2.76–2.90 (m, 2H, C*H_2*Ph), 3.68 [3.70] (s, 3H, O*Me*), 3.76 (q, $J_{CH,Me}$ = 7.2 Hz, 1H, C*H*Me), 3.96–4.12 (m, 1H, H-4), 4.94 (dd, $J_{5,6}$ = 8.6, $J_{4,5}$ = 5.2 Hz, 1H, H-5), 5.84 (dd, $J_{5,6}$ = 8.6, $J_{2,6}$ = 1.5 Hz, 1H, H-6), 6.72 [6.74] (d, $J_{2,6}$ = 1.5 Hz, 1H, H-2), 7.14–7.56 (m, 9H, phenyl hydrogens). The ratio of the two sets of resonances was about 3:2 with the δ values for the minor set of resonances when present shown in square brackets.

General Method for the Preparation of 2-Methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetic Acids ***8a–g***

Aqueous NaOH (10 ml of a 1% w/v solution, 0.75 mmol) was added dropwise to a solution of the respective methyl acetate ester (**7**, 0.75 mmol) in EtOH-H_2O (4:1, *v/v*, 12.5 ml) at 25 °C with stirring. The reaction was allowed to proceed with stirring until TLC (0.25 mm thickness silica gel G microplate) indicated that the reaction was completed (about 2 h). Removal of the solvent *in vacuo*, addition of water (10 ml), and acidification with 5N HCl gave a yellow solid which was filtered off and dried in a drying pistol to afford the respective acetic acid products **8a–g** as a mixture of four diastereomers (see Table 1 for R^1-, R^2- and R^3-substituents, mp and % yield data). The acetic acid products **8a–d**, **8f** were characterized by reconversion to their respective methyl ester derivatives (**7**) in quantitative yield by addition of a solution of excess diazomethane in MeOH at 25 °C with stirring. Infrared (IR) and ^{1}H NMR spectral data for **8a–g** are qualitatively similar except for differences with respect to the R^1-, R^2- and R^3-substituents. Representative IR and ^{1}H NMR spectral data for **8b**, **8c** and **8g** are listed below.

2-Methyl-2-[1-(3-benzoyl-4-para-chlorophenyl-1,4-dihydropyridyl)]acetic Acid ***8b***

IR (KBr): ν = 3057 cm^{-1} (OH), 1802 (CO_2), 1679 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 1.48 [1.56] (d, $J_{Me,CH}$ = 7.2 Hz, 3H, CH*Me*), 3.95 (q, $J_{CH,Me}$ = 7.2 Hz, 1H, C*H*Me), 4.80 (d, $J_{4,5}$ = 4.9 Hz, 1H, H-4), 5.05 (5.08) (dd, $J_{5,6}$ = 7.1, $J_{4,5}$ = 4.9 Hz, 1H, H-5), 6.00 [6.02] (d, $J_{5,6}$ = 7.1 Hz, 1H, H-6), 6.70 (br s, 1H, O*H*), 6.99 [6.96] (s, 1H, H-2), 7.10–7.70 (m, 9H, phenyl hydrogens). The ratio of the two sets of resonances was about 1:1 with the δ values for the second set of resonances when present shown in square brackets.

2-Methyl-2-[1-(3-benzoyl-4-cyclohexyl-1,4-dihydropyridyl)]acetic Acid ***8c***

IR (KBr): ν = 3057 cm^{-1} (OH), 1802 (CO_2), 1671 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 0.98-2.00 (m, 13H, cyclohexyl H-2, H-3, H-4, H-5, H-6, CH*Me*), 2.62–2.68 (m, 1H, cyclohexyl H-1), 3.45–3.60 (m, 1H, H-4), 4.16 [4.00] (q, $J_{CH,Me}$ = 7.1 Hz, 1H, C*H*Me), 5.62 [5.56] (dd, $J_{5,6}$ = 8.7, $J_{4,5}$ = 4.5 Hz, 1H, H-5), 6.72 (br s, 1H, O*H*), 6.96 (d, $J_{5,6}$ = 8.7 Hz, 1H, H-6), 7.30–7.65 (m, 6H, phenyl hydrogens, H-2). The ratio of the two sets of resonances was about 1:1. The δ values for the second set of resonances when present are shown in square brackets.

2-Methyl-2-[1-(3-benzoyl-4-isobutyl-1,4-dihydropyridyl)]acetic Acid ***8g***

IR (KBr): ν = 3080 cm^{-1} (OH), 1802 (CO_2), 1679 (C=O); ^{1}H NMR (Me_2SO-d_6): δ 0.80–1.00 (m, 6H, CH*Me_2*), 1.18–1.44 (m, 5H, CHC*H_2*, CH*Me*), 1.68–1.82 (m, 1H, CH_2C*H*Me_2), 3.54–3.60 (m, 1H, H-4), 4.24 (q, $J_{CH,Me}$ = 6.9 Hz, 1H, C*H*Me), 4.98 [5.02] (dd, $J_{5,6}$ = 7.1, $J_{4,5}$ = 4.8 Hz, 1H, H-5), 6.08 (d, $J_{5,6}$ = 7.1 Hz, 1H, H-6), 6.90 [6.92] (s, 1H, H-2), 7.36–7.52 (m, 5H, phenyl hydrogens). The ratio of the two sets of resonances was about 1:1. The δ values for the second set of resonances when present are shown in square brackets.

General Method for the Preparation of 2-Methyl-2-[1-(3-benzoyl-4-substituted-1,4-dihydropyridyl)]acetamides ***9a–g***

A saturated solution of ammonia in MeOH (10 ml) was added to a solution of the methyl acetate ester (**7**, 2.7 mmol) in MeOH (20 ml), the reaction flask was sealed with a rubber septum, and the reaction was allowed to proceed for 48 h at 25 °C with stirring. The solvent was removed *in vacuo* and the respective product was purified by silica gel TLC using EtOAc-hexane (3:1, *v/v*) as development solvent. Extraction of the band containing the product using EtOAc (2 × 100 ml) and removal of the solvent *in vacuo* afforded the respective product (**9a–g**) as a mixture of four diastereomers [see Table 1 for R^1-, R^2- and R^3-substituents, mp, % yield and Exact Mass (high resolution mass spectral) data]. Infrared (IR) and ^{1}H NMR spectral data for **9a–g** are qualitatively similar except for differences with respect to the R^1- and R^2-substituents. Representative IR and ^{1}H NMR spectral data for **9b** and **9d** are listed below.

2-Methyl-2-[1-(3-benzoyl-4-para-tolyl-1,4-dihydropyridyl)]acetamide ***9b***

IR (KBr): ν = 3312 cm^{-1} (NH_2), 3180 (NH_2), 1720 (CONH), 1679 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 1.36 (d, $J_{Me,CH}$ = 6.6 Hz, 3H, CH*Me*), 2.28 (s, 3H, -C_6H_4-*Me*), 3.82 (q, $J_{CH,Me}$ = 6.6 Hz, 1H, C*H*Me), 4.78 (d, $J_{4,5}$ = 4.8 Hz, 1H, H-4), 5.10 (dd, $J_{5,6}$ = 7.8, $J_{4,5}$ = 4.8 Hz, 1H, H-5), 5.92 (d, $J_{5,6}$ = 7.8 Hz, 1H, H-6), 5.96 and 6.36 (two s, 1H each, N*H_2*), 7.00 (s, 1H, H-2), 7.06–7.50 (m, 9H, phenyl hydrogens).

2-Methyl-2-[1-(3-benzoyl-4-benzyl-1,4-dihydropyridyl)]acetamide ***9d***

IR (KBr): ν = 3328 cm^{-1} (NH_2), 3180 (NH_2), 1720 (CONH), 1687 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 1.24 [1.28] (d, $J_{Me,CH}$ = 7.5 Hz, 3H, CH*Me*), 2.66 (dd, J_{gem} = 14.2, J_{vic} = 2.9 Hz, 1H, CH*H'*Ph), 3.16 (dd, J_{gem} = 14.2, J_{vic} = 5.5 Hz, 1H, C*H*H'Ph), 3.52 [3.60] (q, $J_{CH,Me}$ = 7.5 Hz, 1H, C*H*Me), 4.20–4.26 (m, 1H, H-4), 4.66 and 4.70 (two s, 1H total, N*H*), 5.00–5.10 (m, 1H, H-5), 5.34 (s, 1H, N*H*), 5.80 [5.83] (dd, $J_{5,6}$ = 7.2, $J_{2,6}$ = 1.5 Hz, 1H, H-6), 6.62 [6.72] (d, $J_{2,6}$ = 1.5 Hz, 1H, H-2), 7.10–7.62 (m, 10H, phenyl hydrogens). The ratio of the two sets of resonances was about 1:1. The δ values for the second set of resonances when present are shown in square brackets.

*Methyl 2-Methyl-2-[1-(4-phenyl-5-benzoyl-1,2,3,4-tetrahydropyridyl)]-acetate **10***

10% Palladium-on-charcoal (20 mg) was added cautiously to a solution of **7a** (0.5 g, 1.4 mmol) in EtOAc (10 ml) in a pressure bottle, and the reaction was allowed to proceed in the presence of hydrogen gas at 30 psi with shaking for 24 h at 25 °C. Filtration of the mixture, and removal of the solvent *in vacuo* gave a yellow oil that was purified by preparative TLC using EtOAc-hexane (1:1, v/v) as development solvent to afford **10** (mixture of four diastereomers) as an oil (R_f = 0.65, 200 mg, 47%); IR (film): IR (KBr): ν = 1745 cm^{-1} (CO_2), 1679 (C=O); ^{1}H NMR ($CHCl_3$-d_1): δ 1.44 [1.42] (d, $J_{Me,CH}$ = 7.2 Hz, 3H, CH*Me*), 1.90–2.15 (m, 2H, H-3), 2.90–3.18 (m, 2H, H-2), 3.74 [3.76] s, 3H, O*Me*), 3.88–4.02 (two overlapping q, $J_{CH,Me}$ = 7.2 Hz, 1H, C*H*Me), 4.32–4.40 (m, 1H, H-4), 7.18–7.65 (m, 11H, phenyl hydrogens, H-6). The ratio of the two sets of resonances was about 1:1. The δ values for the second set of resonances when present are shown in square brackets.

*Determination of the pK_a Value for 2-Methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetic Acid **8a***

The pKa, calculated using the Henderson-Hasselebach equation {pK_a = pH + log [HA] / log [A^-]} using the method of Albert and Searjent[10] for **8a** was 9.17 ± 0.01 (n = 5).

Ulcerogenicity Assay

Six male Sprague-Dawley rats weighing 100–120 g, fasted for 24 h, were sacrificed 8 h after oral administration of the test compound (100–1200 mg/kg po dose). The stomach, sternum, and duodenum were removed for macroscopic and microscopic assessment to determine the presence, or absence, of lesions which were used to calculate the UD_{50} value which is defined as the dose of test compound causing lesions in 50% of animals. A chronic ulcerogenesis assay was performed for **8a** by administering a 600 mg/kg po dose twice daily for six days.

In Vitro Stability in Rat Plasma, or Rat Liver Homgenate

Rat liver homogenate (1:3, *w/v*) was prepared by homogenizing whole liver from a Sprague-Dawley rat with phosphate buffer (pH = 7.4). Rat plasma was obtained by centrifugation of whole rat blood, obtained from Sprague-Dawley rats, at 3,000 rpm for 15 min, and then removal of the plasma using a pipet. Standard solutions of methyl 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetate (**7a**), and 2-methyl-2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetamide (**9a**), were prepared by dissolving the test compound (8.5–13.7 mg) in EtOH (5 ml). An aliquot (100 µl) of the standard solution (**7a** or **9a**) was added to either rat plasma (2 ml), or rat liver homogenate (3 ml) and the mixture was incubated at 37 °C with stirring. At selected time intervals (2, 5, 10, 30 min, 1, 1.5, 2, 3, 4.5, 6, 8, 23 h), an aliquot of the incubation mixture (100 µl) was withdrawn, mixed with ice-cold MeCN (400 µl), and stored at –20 °C prior to HPLC analysis. Quantitative HPLC analysis (concentrations were determined using Water's Millennium 2010 software) was performed using a Millipore 3.9 × 150 mm reverse phase C-18 column with MeCN:0.005 M aqueous tetrabutyl ammonium hydrogen phosphate buffer (0.45:0.55, *v/v*) as the mobile phase at a flow rate of 1 ml/min, and UV detection at 205 nm. The retention times for **7a**, **9a**, the acetic acid hydrolysis product **8a**, and the internal standard methyl 2-[1-(3-benzoyl-4-phenyl-1,4-dihydropyridyl)]acetic acid were 9.6, 2.7, 4.0, and 6.6 min, respectively.

In Vivo Inhibition of Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2)

Male Sprague-Dawley rats, 100–150 g in weight, were fed standard laboratory chow and tap water *ad libitum*. All experimental protocols were approved by the Animal Care Committee of the University of Calgary. *In vivo* assessment of COX-1/COX-2 inhibitory activity was determined based on a modification of a previously reported method[15]. Rats were divided into three groups (3 animals in each group). The rats received via oral gavage either vehicle (10% *w/v* acacia), **1a** or **7a** (100 mg/kg po). Test compounds (**1a**, **7a**) were prepared by grinding the appropriate weight with acacia first, and then adding distilled water to form a suspension. The ratio of the acacia to distilled water used was 10% *w/v*.

Animals were anesthetized with pentobarbital 3 h after administration of the test compound. Blood samples (2 ml) were taken from the descending aorta and divided into two samples. The first aliquot (1 ml) was transferred into a glass tube and placed in a water bath at 37 °C. After 45 min, 10 µg of indomethacin, prepared as a 0.1 mg/ml solution in $NaHCO_3$ (1.25% w/v), was added (100 µl) and gently mixed. When aggregating, platelets release thromboxane A_2, which is synthesized via the enzyme cyclooxygenase (COX-1). The production of this enzyme is measured by the release of the stable hydration product, thromboxane B_2. This assay can therefore be used to measure systemic inhibition of COX by an NSAID.

The remaining blood was aliquoted (1 ml) and incubated for 15 min with gentle shaking in a water bath at 37 °C. Lipopolysaccaride (5 µg/ml) was added and the incubation was allowed to proceed for an additional 5 h. The incubation was terminated by addition of 100 µl of 0.1 mg/ml indomethacin in 1.25% $NaHCO_3$ solution. Lipopolysaccaride-stimulated COX-2 reactivity represents predominately a COX-2 response primarily from mononuclear cells [15]. Samples were centrifuged at 4 °C for 10 min at 1,000 *g*. Serum was transferred to Eppendorf tubes and stored tat –20 °C for subsequent measurement of prostaglandin E_2 by ELISA.

Analgesic Assay

Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay reported by Fukawa *et al.*[16] as described previously[17].

References

[1] S. A. Agudoawu, S.-H. Yiu, E. E. Knaus, *Can. J. Chem.* **1997**, *75*, 1106–1109.

[2] G. H. Hamor, *Nonsteroidal anti-inflammatory agents* in *Principles of Medicinal Chemistry* (Ed.: W. O. Foye, 2nd edition), Lea and Febiger, Philadelphia, **1981**, chapter 22.

[3] A. Allais, G. Roussecui, R. Deract, J. Benzoni, L. Chifflot, *Eur. J. Med. Chem.* **1974**, *9*, 381–389.

[4] A. Mukherjee, S. C. Lahiri, *Synthesis and pharmacological evaluation of some potential non-steroidal anti-inflammatory agents with low gastric irritancy*, *Medicinal Chemistry Division*, *193rd ACS National Meeting, Abstract No. 43*, **1987**, *Denver, USA, April 5–10.*

[5] D. L. Comins, A. H. Abdullah, *J. Org. Chem.* **1982**, *47*, 4315–4319.

[6] D. L. Comins, E. D. Stroud, J. J. Herrick, *Heterocycles* **1984**, *22*, 151–157.

[7] U. Eisner, J. Kuthan, *Chem. Rev.* **1972**, *42*, 1–42.

[8] H. J. Hofmann, R. Cimiraglia, *FEBS Lett.* **1988**, *241*, 38–40.

[9] H. J. Hofmann, R. Cimiraglia, *J. Mol. Struct. (Theochem.)*, **1990**, *205*, 1–11.

[10] A. Albert, E. P. Serjeant, In *The Determination of Ionization Constants, A Laboratory Manual*, Chapman and Hall, **1984**, p. 14–17.

[11] D. Robinson, *Am. J. Med.* **1983**, *75*, 26–31.

[12] F. Luzzani, G. Colombo, P. Shiatti, D. Selva, A. Glasser, *Pharmacol. Res.* **1984**, *16*, 755–763.

[13] T. Shen, *J. Med. Chem.* **1981**, *24*, 1–5.

[14] Y. Nagai, K. Kirio, H. Nakamura, H. Uno, H. Nishimura, *J. Med. Chem.* **1987**, *26*, 222–226.

[15] K. Glaser, M.-L. Sung, K. O'Neill, M. Belfast, D. Hartman, R. Carlson, A. Kreft, D. Kubrak, C. L. Weichman, *Eur. J. Pharmacol.* **1995**, *281*, 107–111.

[16] K. Fukawa, O. Kawano, M. Hibi, N. Misaka, S. Ohba, Y. Hatanaka, *J. Pharmacol. Methods* **1980**, *4*, 251–259.

[17] J. K. Buolamwini, E. E. Knaus, *Drug Design Delivery* **1990**, *7*, 19–31.

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