Synthesis of New 2-{[(Phenoxy or Phenyl)acetyl]amino}benzoic Acid Derivatives as 3α-Hydroxysteroid Dehydrogenase Inhibitors and Potential Antiinflammatory Agents

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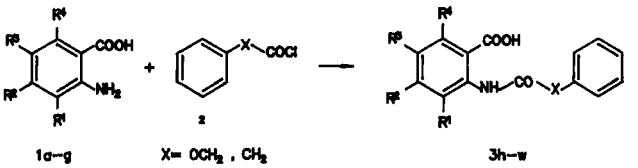
Summary

A number of 2-{[(phenoxy or phenyl)acetyl]amino}benzoic acid derivatives were prepared in about 50% yield from (phenoxy or phenyl)acetyl chloride and anthranilic acid derivatives. All the compounds were tested as *in vitro* inhibitors of 3α-hydroxysteroid dehydrogenase, since enzyme inhibition predicts potential antiinflammatory activity *in vivo* The most active compounds **3 l, m, s** are about 3.5 times more active than acetylsalicylic acid (ASA). Activity is influenced by electronic as well as steric effects.



Compd.*	R^1	R^2	R^3	R^4	Compd.	R^1	R^2	R^3	R^4
1a	H	H	H	H	1e	CH_3	H	H	H
1b	H	Cl	H	H	1f	H	H	H	CH_3
1c	H	H	Cl	H	1g	H	OCH_3	OCH_3	H
1d	Cl	H	Cl	H					

* For identification of substituents in compounds 3h-w see table 1.

Introduction

Continuing our research program on the chemistry and pharmacology of 4(3*H*)-quinazolinone derivatives[1,2], we prepared several 2-{[(phenoxy or phenyl)acetyl]amino}benzoic acid derivatives **3h-w** as intermediates for the synthesis of 3-pyrazolyl-4(3*H*)-quinazolinones funtionalized at the 2-position with a phenoxymethyl or a benzyl group.

A review of literature in this field revealed that several *N*-substituted phenoxyacetamides show a large range of biological activities such as immunosupressive[3], antiviral[4], antimicrobial[5,6], and hypoglycemic [7] activity. Prostaglandin synthetase inhibiting activity is reported [8–10] for a large number of 2-{[(phenoxy or phenyl)acetyl]amino}benzoic acid of type **3h-p.** Some of these compounds showed a better activity than ASA in preliminary antiinflammatory tests in rats.

In consideration of the above biological properties and in order to contribute to a better knowledge of SAR for this class of compounds, we thought that an evaluation of potential antiinflammatory activity might be of interest.

Compounds **3q-w** were also tested to verify if the phenoxy group plays any role on the activity under study.

Lastly, due to the reported antimicrobial activity of phenoxyacetamides [5,6], the compounds **3h-w** were tested against representative gram-positive and gram-negative bacteria as well as against yeasts.

Chemistry

Compounds **3** were obtained very easily by reacting anthranilic acid derivatives **1** with the appropriate chloride **2** (see Scheme 1). Their structure were assigned on the basis of their satisfactory analytical and spectroscopic data .

Biochemistry

The rapid spectrophotometric assay developed by Penning et al. was employed to evaluate the antiinflammatory activity of the compounds **3.** The assay is based on the observation that 3α-hydroxysteroid dehydrogenase (3 α-HSD) of rat liver cytosol can be inhibited *in vitro* by the major classes of non-steroidal antiinflammatory drugs, including the 2-phenylaminobenzoic acid derivatives, in order of their own therapeutic potency [11–15].

Microbiology

The compounds **3h-w** were evaluated for their *in vitro* growth inhibiting activity against gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and gram-positive (*Staphylococcus aureus* ATCC 25923) bacteria and the yeasts *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803.

Biological Results and Discussion

Percentage inhibition of 3α-HSD enzymatic activity is reported in Table 2 for all tested compounds at 0.5 mM or 0.25 mM concentrations. Compounds **3i, p, r, w** were tested at 0.25 mM owing to low solubility of **3i,r** and excessive absorbance at 340 nm of 0.5 mM solutions of **3p, w.** Compound **3l** was also tested at 0.25 mM for comparison.

In both series the highest enzyme inhibition was shown by compounds **3i,l,m** and **3r,s,t** which bear one or two chlorine atoms in the anthranilic moiety, whereas the methyl derivatives **3n,3u** were somewhat less active than the chloro derivatives. Activity dramatically decreased when the anthranilic

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Table 1. Physical data of 2-{[(phenoxy or phenyl)acetyl]amino}benzoic acid derivatives.

Compd.	R^1	R^2	R^3	R^4	X	Cryst. solvent	Mp ° C	Molecular formula	Anal.
3h	H	H	H	H	CH_2O	EtOH	198–200[a]	—	—
3i	H	Cl	H	H	CH_2O	DMF	256–257[b]	—	—
3l	H	H	Cl	H	CH_2O	EtOH	230–231[c]	—	—
3m	Cl	H	Cl	H	CH_2O	EtOH	187–188	$C_{15}H_{11}Cl_2NO_4$	C, H, N
3n	CH_3	H	H	H	CH_2O	EtOH	182–184	$C_{16}H_{15}NO_4$	C, H, N
3o	H	H	H	CH_3	CH_2O	EtOH	191–192	$C_{16}H_{15}NO_4$	C, H, N
3p	H	OCH_3	OCH_3	H	CH_2O	Dioxane	265	$C_{17}H_{17}NO_6$	C, H, N
3q	H	H	H	H	CH_2	EtOH	188–189	—	—
3r	H	Cl	H	H	CH_2	Acetonitrile	165–170	$C_{15}H_{12}ClNO_3$	C, H, N
3s	H	H	Cl	H	CH_2	EtOH	230	$C_{15}H_{12}ClNO_3$	C, H, N
3t	Cl	H	Cl	H	CH_2	Acetonitrile	206–207	$C_{15}H_{11}Cl_2NO_3$	C, H, N
3u	CH_3	H	H	H	CH_2	EtOH	190	$C_{16}H_{15}NO_3$	C, H, N
3v	H	H	H	CH_3	CH_2	EtOH	147	$C_{16}H_{15}NO_3$	C, H, N
3w	H	OCH_3	OCH_3	H	CH_2	EtOH	224–225	$C_{17}H_{17}NO_5$	C, H, N

[a] Ref. [13] 201–203° C; [b] Ref. [13] 248–250° C; [c] Ref. [14] 238–239.5° C.

Table 2. Inhibition of the reduction of 5β-dihydrocortisone and computer calculated values of log *P* [16].

Compd. (0.5 mM)	% Inhibition	log *P*
3h	32.3 ± 3.33	3.345
3i[a]	26.6 ± 1.74	4.164
3l	71.9 ± 2.44	4.164
3l[a]	39.5 ± 1.32	
3m	62.5 ± 0.80	4.059
3n	21.8 ± 1.96	3.344
3o	4.5 ± 0.75	3.844
3p[a]	0	2.778
3q	43.8 ± 1.40	—
3r[a]	27.9 ± 1.58	—
3s	66.0 ± 1.78	—
3t	53.5 ± 1.78	—
3u	19.7 ± 3.40	—
3v	5.8 ± 0.80	—
3w[a]	0	—
ASA	19.0 ± 0.17	—

%Inhibition : arithmetic mean ± standard deviation of three determinations.
[a] Compounds tested at 0.25 mM.

moiety bore a methyl at *ortho* position to carboxy group (compounds **3o,3v**). Lastly, inactive compounds **3p,w** were obtained by 4,5-dimethoxy substitution. Table 2 lists the calculated values of log *P* for all the compound **3** of the phenoxy series. It is worthwhile considering that compounds exhibiting the same lipophilicity have different inhibitory potency and, at the same time, they bear substituents with different σ effects (for the chloro substitution in compound **3i,l**: $\sigma_m = 0.37$, $\sigma_p = 0.23$; for compound **3h,n**: $\sigma_{(CH3)} = -0.07$, $\sigma_H = 0.00$).The phenoxy moiety does not seem to play any particular role for the enzyme inhibitory activity. In fact, the substitution of the phenoxy group by a phenyl group slightly reduced the activity of the chloro derivatives **3l,m**, whereas it increased that of the **3h** derivative. The activity of the methyl and chloro derivatives **3n, i** were left practically unchanged.

Our findings indicate that the electronic effects of substituents influence the enzyme inhibition. On the basis of these results we suggest the hypothesis that the carboxy group is involved in the enzyme binding. In this way, the effect of substituents on the enzyme inhibition could be explained in terms of their electronic influence on the acidic properties of the carboxy group. Moreover, the decreased activity of 6-methyl derivatives **3o,v**, as compared to that of 3-methyl positional isomers **3n,u**, could be justified in terms of the steric hindrance exerted by the 6-methyl substituent on the nearby carboxy group.

Apparently, lipophilicity does not exert a defined effect on the enzyme inhibition. In fact, no coherent change of inhibition activity was observed on comparison of corresponding compounds of the two series in spite of the constant difference in lipophilicity between compounds **3q-w** and **3h-p**, which arises because the lipophilic contribution of a phenyl group is greater than that of a phenoxy group.

Compounds **3l,m,s** were found to be about 3.5 times more active than acetylsalicylic acid and their antiinflammatory activity as well as their ulcerogenic effects will therefore be evaluated *in vivo*.

From an antimicrobial point of view, none of the compounds showed activity against *C. albicans*, *C. tropicalis*, and *P. aeruginosa* at the highest concentration tested (200 μg/ml).

The chloro derivatives **3i,l** were active against *E. coli* and *S. aureus*, showing a minimum inhibitory concentration (MIC) of 50 μg/ml, whereas the remaining compounds were shown to be ineffective against these microorganisms at 200 μg/ml.

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Experimental Part

Chemistry

Melting points (uncorrected): Büchi 530.– IR -spectra: Jasco IR-810 (in nujol).– ^{1}H NMR data: Brücher AC-E 250 MHz ([D$_6$]DMSO, TMS as internal standard).– Elemental analyses: Institut de Chimie Pharmaceutique, Université de Genève.–Compounds **3h**, **i**[17], **3l**[18],**3q**[19] are reported in literature. Their structures were confirmed by IR and ^{1}H NMR data.

General method for the preparation of 2-[[(phenoxy or phenyl)acetyl]amino]benzoic acid derivatives **3h-w**

To a solution of 15 mmoles of the anthranilic acid derivative **1a-g** in 50 ml of acetonitrile (70 ml in the case of **1e,f**) was added the appropriate chloride **2** in equimolar amount. The mixture was refluxed for 1 h and allowed to stand overnight. The solid product was filtered and crystallized from the appropriate solvent, yields 55–75 % (see Table 1).

*2-[(Phenoxyacetyl)amino]-3,5-dichlorobenzoic acid (***3m***)*

IR : ν = 3250 cm^{-1}(NH), 3200–2500 (OH), 1710(CO), 1680 (CO). ^{1}H NMR: δ = 4.70 [s, 2H, CH$_2$], 6.87–7.96 [m, 7H, aromatic H], 10.09 [br s, 1H, exchangeable with D$_2$O, NH], 13.55 [very br s, 1H, exchangeable with D$_2$O, OH].

*2-[(Phenoxyacetyl)amino]-3-methylbenzoic acid (***3n***)*

IR: ν = 3285 cm^{-1}(NH), 3210–2500 (OH), 1700–1650 (2 × CO). ^{1}H NMR: δ = 2.18 [s, 3H, CH$_3$], 4.68 [s, 2H, CH$_2$], 6.96–7.68 [m, 8H, aromatic H], 9.92 [s, 1H, exchangeable with D$_2$O, NH], 13.00 [very br s, 1H, exchangeable with D$_2$O, OH].

*2-[(Phenoxyacetyl)amino]-6-methylbenzoic acid (***3o***)*

IR: ν = 3340 cm^{-1} (NH), 3100–2450 (OH), 1725(CO), 1640 (CO). ^{1}H NMR: δ = 2.43 [s, 3H, CH$_3$], 4.70 [s, 2H, CH$_2$], 6.99–7.42 [m, 8H, aromatic H], 10.55[s, 1H, exchangeable with D$_2$O, NH], 13.85 [very br s, 1H, exchangeable with D$_2$O, OH].

*2-[(Phenoxyacetyl)amino]-4,5-dimethoxybenzoic acid (***3p***)*

IR: ν = 3255 cm^{-1} (NH), 3110–2500 (OH), 1690(CO), 1660–1650 (CO).^{1}H NMR: δ = 3.78 [s, 3H, OCH$_3$], 3.64 [s, 3H, OCH$_3$], 4.72 [s, 2H, CH$_2$], 7.02–8.48 [m, 7H, aromatic H], 12.28 [s, 1H, exchangeable with D$_2$O, NH], 13.50 [very br s, 1H, exchangeable with D$_2$O, OH].

*2-[(Phenylacetyl)amino]-4-chlorobenzoic acid (***3r***)*

IR: ν = 3250–2500 cm^{-1} (NH, OH), 1710(CO), 1660 (CO). ^{1}H NMR: δ = 3.80 [s, 2H, CH$_2$],7.18–8.64 [m, 8H, aromatic H], 11.26 [s, 1H, exchangeable with D$_2$O, NH], 13.87 [very br s, 1H, exchangeable with D$_2$O, OH].

*2-[(Phenylacetyl)amino]-5-chlorobenzoic acid (***3s***)*

IR: ν = 3335 cm^{-1}(NH), 3150–2500 (OH), 1700(CO), 1680–1650(CO). ^{1}H NMR: δ = 3.77 [s, 2H, CH$_2$], 7.28–7.90 [m, 8H, aromatic H], 11.09 [s, 1H, exchangeable with D$_2$O, NH]. OH group was not observed.

*2-[(Phenylacetyl)amino]-3,5-dichlorobenzoic acid (***3t***)*

IR: ν = 3280 cm^{-1} (NH), 3160–2500 (OH), 1690(CO), 1655(CO). ^{1}H NMR: δ = 3.67 [s, 2H, CH$_2$], 7.21–7.91 [m, 7H, aromatic H], 11.08 [s, 1H, exchangeable with D$_2$O, NH], 13.43 [very large, 1H, exchangeable with D$_2$O, OH].

*2-[(Phenylacetyl)amino]-3-methylbenzoic acid (***3u***)*

IR: ν = 3300 cm^{-1}(NH), 3200–2500 (OH), 1690(CO), 1650(CO). ^{1}H NMR: δ = 2.13 [s, 3H, CH$_3$], 3.64 [s, 2H, CH$_2$], 7.18–7.61 [m, 8H, aromatic H], 9.71 [s, 1H, exchangeable with D$_2$O, NH], 12.79 [very br s, 1H, exchangeable with D$_2$O, OH].

*2-[(Phenylacetyl)amino]-6-methylbenzoic acid (***3v***)*

IR: ν = 3200–2550 cm^{-1}(NH and OH), 1700–1650(2 × CO). ^{1}H NMR: δ = 2.36 [s, 3H, CH$_3$], 3.67 [s, 2H, CH$_2$], 7.04–7.54 [m, 8H, aromatic H], 9.82 [s, 1H, exchangeable with D$_2$O, NH], 13.30 [very br s, 1H, exchangeable with D$_2$O, OH].

*2-[(Phenylacetyl)amino]-4,5-dimethoxybenzoic acid (***3w***)*

IR: ν = 3200–2550 cm^{-1} (NH and OH), 1690(CO), 1665(CO). ^{1}H NMR: δ = 3.74–3.79 [superimposed signals, 8H, 2 × OCH$_3$ and CH$_2$], 7.30–8.32 [m, 7H, aromatic H], 11.25 [s, 1H, exchangeable with D$_2$O, NH], 13.40 [very br s, 1H, exchangeable with D$_2$O, OH].

Biological Tests

Biochemistry

3α-HSD activity was determined by using 5β-dihydrocortisone as substrate and β-nicotinamide adenine dinucleotide phosphate, reduced form(β-NADPH), as co-enzyme.

Reduction of 5β-dihydrocortisone was measured at 25° C by monitoring the decrease in absorbance of β-NADPH at 340 nm. The reaction mixture contained 2.5 ml of distilled water, 0.3 ml of 1M potassium phosphate buffer (pH 6.0), 60 µl of 9 mM NADPH, 30 µl of 5 mM 5β-dihydrocortisone and 90 µl of compound solution in the tests or DMSO (used to dissolve compounds) in control experiments.

Each assay was initiated by the addition of 30 µl of a crude preparation of rat liver cytosol [11] and the optical density was followed for 5 min. Enzyme activity was expressed as a variation in optical density decrease per minute (OD minute) and the percentage of inhibition of 3α-HSD was calculated using the following equation:

$$\% \text{ INHIBITION} = (\text{OD min control} - \text{OD min sample}/\text{OD min control}) \times 100$$

Percent inhibition (mean values ± standard deviation of three experiments) of compounds **3h–w** and ASA tested at 0.5 mM or 0.25 mM is reported in Table 2. none of the compounds showed absorbance at 340 nm at these concentrations; moreover the presence at the same time of coenzyme NADPH and substrate 5β-dihydrocortisone were required before the cytosol promoted a change in absorbance.

Microbiology

Antimicrobial tests were carried out by the agar dilution method as described [20].

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