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Pyrrolo[3,2-*c*]pyridine Derivatives as Inhibitors of Platelet Aggregation

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Abstract—A series of pyrrolo[3,2-*c*]pyridines, isosteres of the antithrombotic drug ticlopidine, has been synthesized and evaluated in vitro for the ability to inhibit aggregation of human platelet-rich plasma induced by adenosin 5′-diphosphate (ADP). Structure–activity relationships showed their antiplatelet effects to be related to the lipophilicity.

Platelet aggregation has an important role in the thrombotic events associated to relevant cardiovascular diseases.[1] As a part of our ongoing research directed toward the development of novel antiplatelet agents, we synthesized a series of 4,5,6,7-tetrahydropyrrolo[3,2-*c*]pyridines and evaluated their effects on in vitro platelet aggregation. Pyrrolo[3,2-*c*]pyridine derivatives are isosteres of the currently used thieno[3,2-*c*]pyridine antithrombotic agents, among which ticlopidine (**1**), 5-[(2-chlorophenyl)methyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]-pyridine, and its derivatives (e.g. clopidogrel) have been proven to be effective in the treatment and/or prevention of platelet-dependent disorders,[2] including thrombotic stroke and suppression of platelet activation after coronary stenting[3] (Fig. 1).

Ticlopidine interferes with platelet membrane functions by inhibiting the binding of adenosine 5′-diphosphate (ADP) to its platelet receptors and subsequent platelet-platelet interactions. It also reduces deposition of platelets and fibrin on artificial surface, and prolongs the bleeding time.[4] The effects on platelet functions are irreversible for the life of platelet, as shown by ex vivo measurements of the platelet aggregation inhibition.[5]

We herein report the synthesis, the effects on in vitro ADP-induced platelet aggregation, and preliminary structure–activity relationships of a series of 4,5,6,7-tetrahydropyrrolo[3,2-*c*]pyridines.

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Synthesis

Tetrahydropyrrolo[3,2-*c*]pyridines (**2**–**11**) were synthesised in satisfactory yields as shown in Scheme 1. Compounds **2**, **3**, **5**, **6** and their N(1)-vinyl derivatives (e.g. **4**) were synthesised by applying the Trofimov reaction[6] to 1-benzyl-, 1-ethyl-, 2,5-dimethyl-, and 1,2,5-trimethyl-piperidine-4-one oximes. Vilsmeier-Haack formylation of **6** and 1-vinyl-4,5,6,7-tetrahydro-5-benzylpyrrolo[3,2-*c*]pyridine provided compounds **7** and **4**, respectively. Pyrrolopyridines **9**, **10** and **11** were obtained, respectively, by condensation with ethanolamine and malononitrile, and by reduction with $NaBH_4$ of the formyl derivative **7**. The nitration of **6** under Mencke reaction conditions[7] gave the 2-nitro derivative **8**. The synthesis of compounds **2**–**4** and **10** will be fully described elsewhere, whereas the other derivatives tested in this study were prepared according to reported procedures.[8] As for diastereomeric 4,7-dimethyl substituted pyrrolopyridines **5**–**11**, NMR data indicated that piperidine ring has a semi-chair conformation with *trans* diequatorial 4- and 7-CH_3 groups.[9]

Antiplatelet Effects

Pyrrolo[3,2-*c*]pyridine derivatives **2**–**11** were evaluated as platelet aggregation inhibitors by measuring their effect on the in vitro aggregation of human platelet-rich plasma (PRP) induced by ADP, by using a turbidimetric method.[10] The results are summarized in Table 1. With the exception of 2-nitro- (**8**) and 2-hydroxymethyl-

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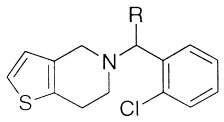


Figure 1. Structure of the antithrombotic agents ticlopidine (**1**, R = H) and clopidogrel (R = $COOCH_3$).

(**11**) 4,5,7-trimethyl-4,5,6,7-tetrahydropyrrole[3,2-*c*]pyridine derivatives, all the test compounds appreciably inhibited the platelet aggregation at doses close to the IC_{50} value of ticlopidine.

Within the limits of this screening, the closest analogue (**2**) of ticlopidine and the 2-formyl derivative **7** showed the highest activities. The aggregation tracing relative to the control revealed a typical biphasic curve. In general, the test compounds inhibited the second phase of in vitro aggregation, whereas the most active ones (e.g. compound **7**) partially inhibited also the primary wave.

Overall, pyrrolo[3,2-*c*]pyridines exhibit a moderate, but structure-dependent, inhibition of in vitro platelet activation, two of them (**2** and **7**) having an activity comparable to that of ticlopidine. Actually, ticlopidine itself is known to display weak platelet inhibitory effects when assayed in vitro at the active concentration attained in vivo.[5] Neverthless, the apparent relationship between the in vitro antiplatelet effect and the in vivo antithrombotic potency, observed for other series of compounds (e.g. 3-carbamoylpiperidines),[11] prompt us to examine further pyrrolopyridine derivatives and to select candidates for in vivo and/or ex vivo activity measurements.

Lipophilicity and Related Parameters

To detect the physicochemical factors possibly related to the inhibition of ADP-mediated platelet aggregation, we undertook an examination of the partitioning behaviour, by calculating 1-octanol/water partition coefficients and measuring retention in RP-HPLC.

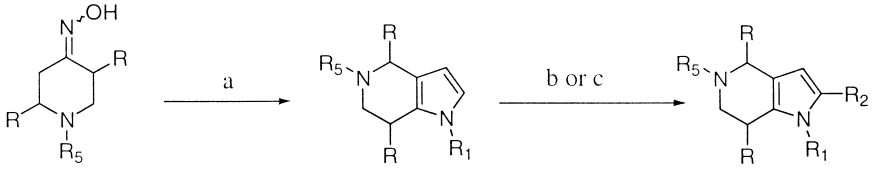
Log *P* values (Table 1), calculated by CLOG P software,[12] based on the fragmental method of Hasch and Leo,[13] indicated that the compounds under examination cover a range of about 4 log units. For a number of compounds, distribution coefficients (log *D*) at physiological pH (7.40), where piperidine nitrogen is mainly in the protonated form, were also measured using the conventional 'shake-flask' (SF) technique.[14]

RP-HPLC retention data were measured using a new silanol-deactivated octadecylsilane (ODS)[15] as the nonpolar stationary phase, at 0.05-increments of the volume fraction of methanol in the aqueous mobile phase (ϕ_{MeOH}). In agreement with previous results,[16,17] the capacity factors (log k') of pyrrole[3,2-*c*]pyridines increased linearly ($r^2 > 0.95$) with decreasing methanol concentration in the mobile phase in the range $0.1 < \phi_{MeOH} < 0.7$. Thus, by using the following linear relationship

$$\log k' = \log k'_w - s\phi$$

log k'_w that is the logarithm of the capacity factor extrapolated to 100% water in the mobile phase, and *s* (slope), that is a constant for the solute-eluent combination, were calculated and reported in Table 1.

Other authors had observed that comparing between them the slope *s* and log k'_w may help in unraveling differences in polarity and H-bonding (HB) properties within a given series of compounds.[18–20] With our compounds, the variations in log k'_w values are more or less those expected from log *P* scale, whereas *s* parameter appeared to be more dependent on the HB capacity of the compounds. In fact, ticlopidine (**1**) and compound **4**, two net HB acceptors, have *s* value of ca. 1.4, whereas



2, 3, 5, 6

8 $R_2 = NO_2$

7 (**4**) $R_2 = CHO$

d → **9** $R_2 = CH{=}NCH_2CH_2OH$

e → **10** $R_2 = CH{=}C(CN)_2$

f → **11** $R_2 = CH_2OH$

Cpd.	R	R_1	R_5
2	H	H	$CH_2C_6H_5$
3	H	H	CH_2CH_3
4	H	$CH{=}CH_2$	$CH_2C_6H_5$
5	CH_3	H	H
6-11	CH_3	H	CH_3

Scheme 1. (a) C_2H_2, KOH, DMSO, 80–90 °C, 4–6 h, 40–50%; (b) **8**: $Cu(NO_3)_2$, Ac_2O, rt 5 h, 47%; (c) **4, 7**: $POCl_3$, DMF, rt, 5 h, 78–80%; (d) $NH_2CH_2CH_2OH$, toluene, reflux, 5 h, 92%; (e) $CH_2(CN)_2$, EtOH, reflux, 3–5 h 95%; (f) $NaBH_4$, EtOH, rt, 5 h, 84%.

Table 1. Antiplatelet activity and physicochemical data of 4,5,6,7-tetrahydropyrrolo[3,2-*c*]pyridine derivatives **1**–**11**

Compound	% Inhibition of platelet aggregation[a]	CLOG P[b]	Log D[c]	RP-HPLC retention data[d]	
				Log k'_w	*s*
Control	6.0 ± 1.1				
1	48.5 ± 5.9***	4.04	>3.00	1.99	1.41
2	46.5 ± 8.9***	2.24	1.59	1.66	4.12
3	23.7 ± 5.0**	1.20		0.60	5.58
4	38.7 ± 0.5***	3.08		1.29	1.42
5	26.0 ± 14.5	1.24	−1.08	0.88	4.40
6	28.5 ± 6.6**	1.91	−0.41	0.96	5.06
7	48.0 ± 7.3***	1.72	0.69	0.93	6.24
8	3.6 ± 1.6	2.13	1.14	0.97	4.77
9	12.2 ± 7.1	−0.05		[f]	
10	ND[e]	1.19		1.39	3.04
11	3.3 ± 3.3	0.87		1.02	7.44

[a]Platelet-rich plasma (PRP) was pre-incubated with the test compounds (250 μM) or with dimethylsulphoxide (0.5%, control) at 37 °C for 4 min. The inducer ADP (10 μM) was then added. Percent inhibition of aggregation is presented as means ± SEM ($n = 4$–6). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; significantly different from the respective control value.
[b]Calculated log *P* values.[12]
[c]1-Octanol/water distribution coefficients measured at pH 7.40 (0.01 MOPS buffer).
[d]Capacity factors extrapolated at 100% water eluent ($\log k'_w$) and slope (*s*) determined on an octadecylsilane stationary phase.[15]
[e]ND = not determined, because of its low solubility.
[f]Due to its hydrophilicity, it was not retained even at highly polar mobile phase (>90% water).

all the others pyrrolopyridine derivatives have $s < 3.0$. The total HB donor capacity (see **5** and **6**) seems to depend essentially on the pyrrole NH, and marginally on the piperidine NH, as assessed by the Abraham's α_2^H descriptors of HB donor capacity (0.41 and 0.06, respectively).[21] The maximum value of *s* parameter (ca. 7.4) observed for **11** is consistent with the dominance of conformations lacking intramolecular H-bond between pyrrole NH and OH group of hydroxymethyl substituent in 2 position.

Interestingly, for the congeners **7** (2-CHO) and **8** (2-NO_2), the log k'_w values are almost identical, whereas the *s* values differ significantly (6.24 and 4.77, respectively). Moreover, the log k'_w and *s* values of compound **7** and the unsubstituted congener **6** are close, suggesting that polarity/polarizability and HB acceptor ability of the nitro substituent should negligibly affect the RP-HPLC retention behaviour. To gain insights into conformations and/or self-association properties of congeners **6**, **7** and **8**, we examined their FT-IR spectra in chloroformic solutions, at concentrations below 5×10^{-2} M. The ν_{NH} absorption at 3479 cm^{-1} for compound **6** was assigned to the stretching of free pyrrole NH. A shift of the stretching vibration band ν_{NH} to lower wavenumber (3445 cm^{-1}, $\Delta\nu_{NH} = 34$ cm^{-1}) for the 2-CHO congener (**7**) could account for an intramolecular HB, NH···O=C, or a dipolar alignment. A smaller $\Delta\nu_{NH}$ (ca. 10 cm^{-1}) found for the nitro derivative **8** revealed a weaker acceptor ability of NO_2 group and probably a lower contribution of the internally H-bonded conformations. A weaker and broader, but concentration-dependent, band at 3270 cm^{-1}, assigned to ν_{NH} vibration of intermolecular (self-associated) hydrogen-bonded species (NH···O=C), was observed in the IR spectrum of **7**, and not in that of the NO_2 congener **8**. This indicated that CHO group is more effective than NO_2 group in increasing the HB donor ability of the pyrrole NH. Most likely, the torsion angle of the nitro group is far from 0°, and this loss of coplanarity could result in a diminished electronic conjugation.[22]

Lipophilicity–Activity Relations

A comparison of antiplatelet effects values with physicochemical parameters suggested trends of correlations. Lipophilicity appears to play a role in modulating the antiplatelet activity (Fig. 2).

In fact, with the exception of less active derivatives **8** and **11**, when lipophilicity increases activity increases as well, until a value of log *P* around 2 is reached. A vinyl group on pyrrole NH (**4**) is not beneficial, and the methyl groups in 4 and 7 positions should not exert any different effect from those accounted for by the lipophilicity. As far as the role of the 2-X substituents is concerned, our results indicated that, besides the additive contribution of the 2-X fragments to lipophilicity, which could explain the low activity of the most hydrophilic congeners **9** and **11**, other factors, especially the electronic and conformational ones, should be taken into account. Thus, the formyl congener (**7**), equiactive with ticlopidine despite its lower log P value, resulted significantly more active than the nitro congener (**8**). The *s* parameter from RP-HPLC and IR spectroscopy revealed that the formyl group (and not the nitro group) influences the ability of NH to act as a HB donor, suggesting a role of the pyrrole NH in modulating the inhibition of platelet aggregation.

In conclusion, our study showed 4,5,6,7-tetrahydropyrrolo[3,2-*c*]pyridines to be inhibitors of ADP-stimulated platelet aggregation in vitro, their activity being related to the lipophilicity. Actually, the importance of

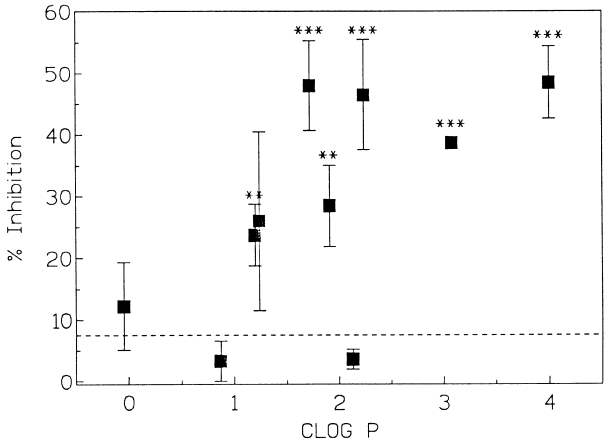


Figure 2. Plot of inhibition of ADP-induced platelet aggregation against lipophilicity as assessed by CLOG P values. The dashed line represents the control inhibition.

lipophilicity in the antiplatelet activity had been demonstrated by others for different series of derivatives.[23] Our results highlight other properties, especially H-bonding, likely involved in the platelet aggregation inhibition of pyrrolo[3,2-*c*]pyridines, and stimulate a deeper examination of further isosteres and analogues of ticlopidine-related compounds.

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