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β-ADRENERGIC and THROMBOXANE A_2 ACTIVITIES OF RECENT MODIFIED TRIMETOQUINOL ANALOGUES

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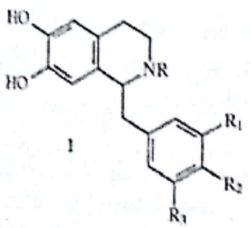
ABSTRACT

Several Trimetoquinol (TMQ) derivatives were prepared and evaluated for their action on both β- adrenergic and thromboxane A_2/prostaglandin H_2 (TP) receptor systems. These particular derivatives explored the importance of TMQ's chiral center, the homologation of previously described N-benzyl derivatives, and various modifications of TMQ's trisubstituted benzyl group to probe receptor affinities. The results of this studies indicated that TMQ's chiral center is important for receptor interactions. Elongation of an N-Benzyl substituent fails to imporove potency, but modifications of the trisubstituted benzyl group has provided a new lead for modified TMQ derivatives with enhanced activity in TP receptor systems.

INTRODUCTION

Trimetoquinol (TMQ, **1**, Figure 1) is a potent, nonselective, nonclassical β-adrenergic agonist (1) and nonprostanoid thromboxane A_2/prostaglandin H_2 (TP) antagonist. This compound contains a trimethoxybenzyl substituent which produces a chiral center that is unique among β-agonists and nonprostanoid TP antagonists. This chiral center shows a strong stereodependence for binding to β-adrenergic (S>>R) and TP (R>>S)[4].

Since TMQ lacks significant receptor subtype selectivity in each system, a planar amidine derivative 2 was prepared to investigate the effect of conformationally restricting the trimethoxybenzyl substituent. The planarity of this amidine derivative will provide insight on the need for a chiral center and may have a substantial impact on receptor potency and selectivity.



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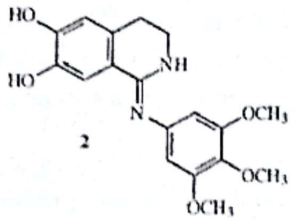

2

1 R=H, R_1=R_2=R_3=OCH_3
3 R=CH_2Ph, R_1=R_2=R_3=OCH_3
4 R=CH_2CH_2Ph, R_1=R_2=R_3=OCH_3
5 R=H, R_1 = I, R_2=R_3=OCH_3
6 R=H, R_1=R_3= I, R_2=OCH_3
7 R=R_3=H, R_1= NO_2 , R_2=OH
8 R=R_1=R_3=H, R_2= NO_2
9 R=R_1=R_3=H, R_2=NH_2

Several attempts were made to improve the biological profile of TMQ by investigating various structural modifications to improve potency and/or receptor subtype selectivity. Most modifications resulted in TMQ analogues which either retained or lost potency as β-agonists or TP antagonists[5,6]. Although many derivatives generally had reduced potency, enhanced receptor subtype selectivity in β-adrenergic (β_2 vs β_1 selectivity) and TP (α-subtype vs τ-subtype) receptor systems was observed for a number of N-benzyl derivatives.[7,8].

In an effort to regain receptor affinity while maintaining receptor subtype selectivity, the N-substituent of N-benzyl TMQ (3) was elongated to a phenylethyl homolog (4) to probe for a potential binding site in β-adrenergic and TP receptors responsible for the receptor subtype selectivity.

The aforementioned trimethoxybenzyl substituent is apparently important for receptor affinity and receptor activation. This substitution is not found in classical β-adrenergic agonists or non prostanoid TP antagonists and may be significant in identifying important portions of the receptors for enhanced ligand affinity and/or function. Seveal TMQ analogue have been prepared but only two iodinated derivatives[9] have exhibited similar or enhanced affinity for human β_1- and β_2-adrenergic and TP receptors[10,11]. Several additional derivatives have been prepared to investigate the structural activity relationships of TMQ analogues as β-adrenergic agonists and TP antagonists.

MATERIAL AND METHODS

β-adrenergic receptor studies: Male albino Hartley guinea pigs weighing 300-400g were used. Isolation of tissues and preparations of spontaneously beating right atria and carbachol (0.3 μM)- contracted tracheal strips

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were done as described previously[7,8]. Cumulative concentration- response curves to each drug were determined. Each successive drug addition was added only after the effects of the previous concentration had reached a maximum response, and the results expressed relative to the maximal response to 10^{-5} M isoproterenol added after the completion of each concentration-response curve.

Platelet Inhibition Studies:

Blood was collected from normal human volunteers who reported to be free of medication for at least 10 days. Platelet- rich plasma (PRP) was prepared and used for studies for inhibition of aggregation and serotonin secretion by drugs in the presence of U46619, a Tp agonist as previously described in our laboratory[6,7].

Platelet aggregation experiments were performed according to the turbidometric method in dual channel aggregometers interfaced to computer for the acquisition, quantification, presentation and management of aggregation data[12]. [^{14}C] Serotonin (0.05 µCi per mL) was added to PRP for 20 min prior to the start of the experiment to allow for uptake of label into dense granules. U46619 was used at the minimum concentration required to stimulate maximal aggregation and serotonin secretion. Aspirin (1 mM) was added to PRP to block the formation of endogenous prostaglandins. After one min incubation of PRP samples with aspirin and drug, U46619 (0.3- 1 µM) was added and the aggregation response was monitored for 4 min. Samples were centrifuged and a 0.1 ml aliquot of the supernatant was mixed into an emulsion- type scintillation mixture and radioactivity was measured by liquid scintillation spectrometry.

Background counting rates (BKG, CPM) were determined from the supernatants of unstimulated samples and was less than 10% of that released into the supernatants by U46619 alone.

The percent secretion of serotonin was calculated by the following formula: percent serotonin = [Sample CPM-BKG CPM) / (Total CPM- BKG CPM] x 100% where CPM = counts per min and Total CPM = amount of radioactivity in the sample.

Radioligand binding studies with Thromboxane A_2/ Prostaglandin H_2 (TP) Receptor Sites in Human Platelets:

For binding experiments, human PRP was centrifuged and resuspended in 50 µM Tris saline buffer, pH 7.2 according to Sin et al[11]. Platelets ($1x10^8$) were incubated with 5 nM [^{3}H] SQ 29, 54 in a final vol of 0.5 mL[13]. Unlabelled SQ 29, 548 (50 µM) was used to dtermine nonspecific binding. Varying concentrations of each drug were used to quantify the inhibition of specific [^{3}H] SQ 29, 548 binding.

Samples were incubated for 30 min at room temperature, and rapidly filtered by vacuum through Whatman GF/B glass fiber filters on a brandel cell harvester and washed for 10 sec with ice cold TIRIS saline buffer. Filters were placed in plastic scintillation vials containing 10 mL of an emulstion-type scintillation mixture and radioactivity was meausred by liquid scintillation spectrometry. Specific binding to human platelets varied between 88-95%.

RESULTS AND DISCUSSION

Each of the TMQ analogues has been evaluated for β-adrenergic and TP receptor activities. β-Adrenergic evaluation was performed in guinea pig atria (β_1) and tracheal (β_2) strips to evaluate increased heart rate and smooth muscle relaxant properties, respectively. TP antagonist properties (α-subtype) were determined from the ability of these analogues to inhibit agonist (U 46619) induced platelet activation (aggregation and serotonin secretion) and to inhibit [^{3}H] SQ 29, binding to TP receptors.

The biological profile of amidine-TMQ **2** supports the stereoselective interactions of TMQ with each receptor system.

This planar analogue is a very weak β_2 agonist as compared to TMQ (Table 1). Since this compound was such a weak β_2 agonist, the β_1 properties were not evaluated. This compound also failed to show TP antagonism at a 400 µM dose (Table 2).

Table 1. Comparative adrenergic agonist activities of trimetoquinol analogues on guinea pig right atrial (β_1) and tracheal (β_2) Tissues

Compound	β_1 (n=3-5)pD_2*	β_2 (n=4-9) pD_2*
(S)-1	8.35 ± 0.13	8.80 ± 0.14
(R)-1	5.84 ± 0.18	5.76 ± 0.05
2	ND	3.95 ± 0.12
4	4.62 ± 0.36	4.64 ± 0.23
7	5.91 ± 0.31	6.55 ± 0.15
8	7.26 ± 0.26	7.49 ± 0.12
9	6.12 ± 0.04	6.75 ± 0.10

* pD_2= - log EC_{50}
ND = not determined

Table 2. Comparative potencies of trimetoquinol derivatives for the inhibition of U46619 (1 μM) Induced Human Platelet Activation (Aggregation and serotonin secretion) and (H^3) SQ 29 , 548 binding (5 nM) to Tp receptros

Compound	Platelet aggregation pIC_{50}*	serotonin secretion pIC_{50}*	Binding[H^3] pIC_{50}*	SQ 29, 548 pK_i*
1	5.94 ± 0.21	5.88 ± 0.13	6.35 ± 0.10	6.76 ± 0.09
2	3.70 ± 0.12	3.69 ± 0.19	3.56 ± 0.03	3.92 ± 0.07
4	4.42 ± 0.12	4.46 ± 0.36	4.38 ± 0.18	4.79 ± 0.18
7	5.90 ± 0.10	5.95 ± 0.19	6.06 ± 0.19	6.47 ± 0.19
8	4.35 ± 0.05	4.22 ± 0.07	4.60 ± 0.15	5.02 ± 0.15
9	3.89 ± 0.04	3.84 ± 0.04	4.05 ± 0.04	4.46 ± 0.04

* $pIC_{50} = -\log IC_{50}$

* $pK_i = -\log K_i$

The N-phenylethyl derivative **4** was a weak agonist in both β_1 and β_2 adrenergic systems (Table 1). This derviative was slightly more potent than the N-benzyl derivative **3** on β_1 tissue, but less potent on β_2 tissues[5].

Unlike the N-benzyl derivative which has β-receptor subtype selectivity[5], N-phenylethyl derivative is devoid of any β-receptor subtype selectivity. In TP receptor systems, compound 4 is slightly less potent in preventing U46619-induced platelet aggregation in comparison to the N-benzyl derivative **3**, but it is slightly more potent in preventing serotonin secretion (Table 2).

The modification of the 1-benzyl substituent provided derivatives **7,8** and **9** with β-adrenergic agonist activity (Table 1) comparable to previously synthesized iodo-TMQ derivaties **5** and **6**[14]. The rank order of potency of increased heart rate (β_1) and tracheal smooth muscle (β_2) relaxation in comparison to the TMQ isomers is (S) - (-) - TMQ > nitrophenyl **8** > aniline **9** > nitrophenol **7** > (R)- (+) - TMQ.

In comparison to (S)- (-) - TMQ, each of these derivatives failed to show receptor subtype selectivity. The modified benzyl derivatives, **7,8** and **9** were also concentration dependent TP antagonists (Table 2). The rank order of potency of TP antagonist properties for the inhibition of platelet aggregation and serotonin secretion is (±) - TMQ = nitrophenol **7** >> nitrophenyl **8** > aniline **9**. The modification of the 1-benzyl substituent to the nitrophenol derivative **7** provided significant potency for the inhibition of platelet aggregation (α-subtype) and serotonin secretion that is comparable to previously[14] synthesized iodo-TMQ derivatives **5** and **6**.

Further studies with rat vascular aorta (τ-subtype) are planned to investigate potential receptor subtype seletivity.

CONCLUSION

Amidine - TMQ **2** is considerably less active than TMQ in both β-adrenergic and TP receptor systems. Thus, the conversion of the chiral center of TMQ **1** into a planar amidine provides additional support for the importance of the chiral center for the stereoselective interactions of TMQ with the appropriate receptor systems.

N-Phenylethyl -TMQ **4** has similar activity to the previously reported N-benzyl derivative **3**, but provides no significant improvement of the biological profile in either β-adrenergic or TP receptor systems.

Several racemic 1-benzyl derivatives **7-9** have been evaluated in both β-adrenergic and TP receptor systems. The increased potency of nitrophenol **7** in a TP receptor system with a concurrent loss of activity in β-adrenergic receptor systems constitutes this compound as a potential new lead compound to optimize TP antagonist properties. This compund's increased potency may be due to an ionic interaction between the phenolic hydroxyl group and a complementary cationic group on the TP receptor. The lack of significant potency for aniline **9** rules out potential hydrogen bonding interactions of nitrophenol **7** with this receptors.

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تأثيرات شبيهات حديثة للترايميتوكونول على مستقبلات البيتا الأدرينالينية ومستقبلات الثرمبوكسان أ٢

جمال الدين أمين شمس

قسم الفارماكولوجى - كلية الطب البيطرى

جامعة الزقازيق - مصر

لقد أثبتت التجارب أهمية ذرة الكربون المركزية لمركب الترايميتوكوينل والتى تميزه إلى شبيهين وأيضا أهمية مجموعة البنزيل فى تحديد عديد من المستقبلات حيث أنه وجد أن ذرة الكربون المركزية بمركب الترايميتوكوينول لها أهمية فى تفاعل المركب مع عديد من المستقبلات وأن استطالة المجموعات الكيميائية على ذرة النيتروجين تفقد الترايميتوكوينول قوته للتفاعل مع مستقبلات البيتا الأدرينالينية ومستقبلات الثرومبوكسان أ٢ ولكن بعض التحورات التى تمت فى مجموعة البنزيل أدت الى انتاج مركبات قوية كمثبطة لمستقبلات الثرومبوكسان.