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Microwave-assisted synthesis of 5-aryl-3-hydroxy-2-oxindole derivatives and evaluation of their antiglaucomic activity

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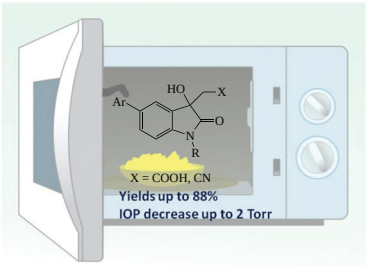
A set of new 5-aryl-3-hydroxy-2-oxindoles was synthesized by decarboxylative condensation of the corresponding 5-aryl-substituted isatins with malonic or cyanoacetic acids under microwave irradiation. The antiglaucomic activity of the obtained compounds was evaluated. The most water soluble compounds can reduce the intraocular pressure (IOP) up to 2 Torr.



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Antiglaucomic drugs available on the market belong mainly to six different classes: prostaglandin analogs, carbonic anhydrase inhibitors, beta-blockers, alpha-agonists, miotics, and rho-kinase (ROCK) inhibitors.[1–4] However, pharmacotherapy of glaucoma is still severely limited due to cases of individual intolerance or tachyphylaxis.[5] Thus, the search for antiglaucomic drugs that interact with another molecular target remains an important and urgent task.

It is known that the endogenous hormone melatonin, in addition to regulating circadian cycle, has antioxidant properties and participates in the regulation of intraocular pressure (IOP).[6] The hypotensive properties of melatonin are associated with its effect on the melatonin MT3-subtype receptor (known as NRH:quinone oxidoreductase 2, NQO2, QR2), which means that NQO2 inhibitors can become a new generation of antiglaucomic drugs.[7,8]

The modification of the position 3 of the oxindole core is a simple way of obtaining compounds with a wide range of biological activity.[9] The starting compound for this modification can be either 2-oxindole[10] or isatin,[11,12] but only with isatin it becomes possible to retain the oxygen in the position 3 and to obtain 3-hydroxy-2-oxindole derivatives. A set of 3-hydroxy-2-oxindoles with various substituents was prepared using decarboxylative aldol condensation of the corresponding isatins with cyanoacetic and malonic acids, and the ability of these compounds to normalize IOP was studied in normotensive rabbits.[13–16] One of the most promising compounds was 2-(1-benzyl-3-hydroxy-5-methoxy-2-oxoindolin-3-yl)acetonitrile **A**, capable of reducing IOP by 5.6 Torr from baseline during *in vivo* experiments[14] (Figure 1). In addition, it was found through *in silico* experiments that the enhancement of the biological response may be associated with an increase in π–π stacking between compound **A** and the cofactor of the NQO2 enzyme, flavin adenine dinucleotide (FAD). These assumptions were made from studying the binding of 3-hydroxy-2-oxindoles to the active site of the NQO2 enzyme by molecular modeling in the AutoDock4 molecular docking program. Thus, the aryl fragment at position 1 of compound **A** may be the key functional group for the binding between the enzyme and ligand.

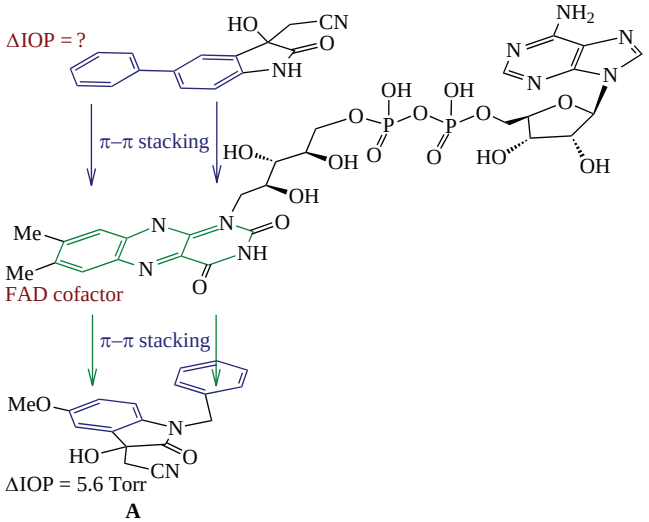


Figure 1 Proposed increase in binding affinity due to π–π interaction with FAD cofactor in NQO2 active site.

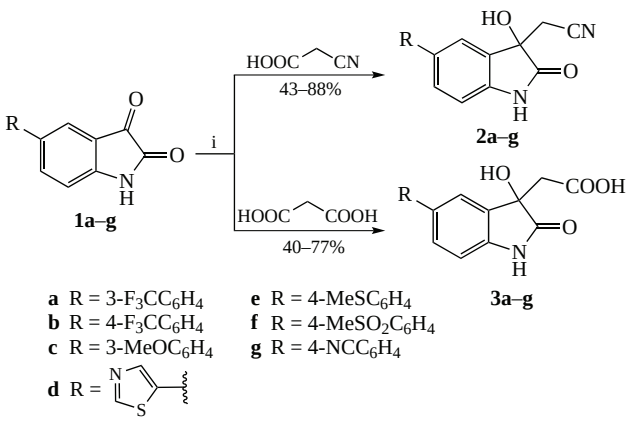
In this work, we synthesized a group of compounds with an aryl fragment in the position 5 of the 2-oxindole ring. This fragment should perform several important functions: it affects the hydrophilic–lipophilic balance of the molecule, fills the hydrophobic pocket of the active site of the NQO2 enzyme more densely, displacing water from it, and provides additional π–π interactions between the ligand, the FAD cofactor and the aryl fragments of amino acids in the active site. 5-Aryl-substituted isatins **1a**–**g** were chosen as starting materials.[17] The modification of the position 3 of the 2-oxindole core was carried out by the express microwave-assisted synthesis of 3-hydroxy-2-oxindoles mentioned above.[13] In fact, condensation of isatins **1a**–**g** with cyanoacetic or malonic acids in 1,4-dioxane in the presence of triethylamine in domestic microwave oven (260 W, 5 min) led to 3-hydroxy-3-(cyanomethyl)oxindoles **2a**–**g** or 3-hydroxy-3-(carboxymethyl)oxindoles **3a**–**g**, respectively (Scheme 1).

Compounds **2a**–**g** and **3a**–**g** can be considered as bioisosteres of melatonin, thus they may serve as potential ligands of the MT3 melatonin receptor subtype. To confirm this, the molecular modeling was carried out using the AutoDock v.4.2.5.1 software package. A model of the NQO2 enzyme with a melatonin molecule in the active site was used (PDB code 2QWX, 1.5 Å resolution). The ligand efficiency (LE) and the concentration of half-maximal inhibition (IC_{50}) were calculated from the estimated minimum binding energy of the ligand with the enzyme (ΔG_m) according to known formulas (see Online Supplementary Materials). Differences in the ΔG_m for the *R* and *S* enantiomers of the ligand are usually insignificant. Moreover, ΔG_m of *S*-isomer is usually slightly less, *e.g.* for compound **2c** $\Delta G_m(S) = -8.36$ kcal mol^{-1} and $\Delta G_m(R) = -8.16$ kcal mol^{-1}.

From the data obtained, it can be unambiguously concluded that the cyano group in the 3-positioned substituent of the 2-oxindole core is preferable to the carboxy group. This is confirmed by our experimental data published earlier.[13] Of the above compounds, special attention should be paid to compounds **2e** and **2g**, as they have the maximum (in modulus) ligand efficiency.

After promising results of molecular modeling, the biological activity of all obtained compounds was tested *in vitro* on NQO2 enzyme and *in vivo* on normotensive rabbits (Table 1). The dependence of IOP reduction on time after single instillation of **2d** and **2f** in buffer solution is shown (Figure 2).

Unfortunately, poor solubility of most compounds in phosphate buffer solution, affected by the addition of a large hydrophobic fragment to the position 5 of the 2-oxindole core, made it impossible to evaluate their effectiveness in reducing IOPs in normotensive rabbits. In addition, since maximum inhibition at a concentration of 10 μM does not exceed 22%, none of the obtained compounds appear to be inhibitors of the NQO2 enzyme.



Scheme 1 *Reagents and conditions*: i, Et_3N, dioxane, MW, 260 W, 5 min.

Table 1 IC_{50} values and inhibition activity of 5-aryl-3-hydroxy-2-oxindole derivatives.

Compound	NQO2 inhibition at 10 μM (%) (n = 3)	Max IOP reduction/ Torr
2a	20.3	ins.[a]
2b	1.1	ins.
2c	21.7	ins.
2d	9.1	2.00
2e	4.7	ins.
2f	10.5	1.40
2g	2.0	ins.
3a	–0.8	ins.
3b	2.4	ins.
3c	2.5	ins.
3d	7.9	ins.
3e	4.2	ins.
3f	9.1	ins.
3g	5.4	ins.
Quercetin	98.3 (IC_{50} 0.08 ± 0.02 μM)	n.t.
Melatonin	85.2 (IC_{50} 63.5 ± 26.7 μM)	2.71
Timolol	n.t.[b]	3.00

[a] ins. – insoluble in phosphate buffer; [b] n.t. – not tested.

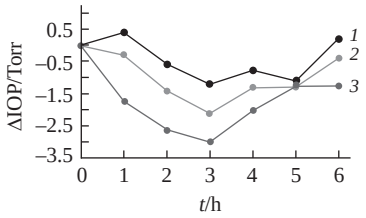


Figure 2 Graphical representation of IOP reduction after instillation of 50 μl of 0.1% w/v solution of (*1*) compound **2f**, (*2*) compound **2d** and (*3*) Timolol as the reference.

In conclusion, fourteen new 5-aryl-3-hydroxy-2-oxindole derivatives were synthesized using the MW-assisted decarboxylative condensation of substituted isatins with malonic and cyanoacetic acids. Prediction of the IC_{50} and LE of the obtained compounds towards the melatonin MT3-subtype receptor (enzyme NQO2) was carried out *in silico* by molecular docking (AutoDock 4). The data obtained suggest that 5-aryl-3-hydroxy-2-oxindole derivatives may be good ligands for the NQO2 enzyme with IC_{50} lower than 0.01 μM. However, when the inhibitory activity of synthesized compounds against quinone reductase 2 (possible molecular target for IOP reduction) was studied *in vitro*, it turned out that none of them showed inhibition higher than 22% at a concentration of 10 μM. The impact of new compounds on IOP was studied *in vivo* on normotensive rabbits. Due to the low solubility in phosphate buffer solution, data were obtained only for two compounds whose ability to reduce IOP did not exceed melatonin (2.71 Torr).

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.06.035.

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