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Soft cannabinoid analogues as potential anti-glaucoma agents

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Cannabinoids have intraocular pressure (IOP) lowering effects, thus, they have a therapeutic potential in the treatment of glaucoma. Unfortunately, in the same time, they show CNS and cardiovascular effects as well. Our aim was to develop a safer, cannabinoid type anti-glaucoma agent, a topically applied soft analogue, that has local, but no systemic effect. The lead compound chosen was a nitrogen-containing cannabinoid analogue that was shown to have IOP lowering activity. A full library of possible soft drugs was generated and the structures were ranked based on the closeness of calculated properties to those of the lead compound. The lead compound has been synthesized, and a preliminary pharmacological study was performed. The structure-activity relationship and pharmacological results indicate a good possibility for the development of a safe, soft anti-glaucoma agent.

1. Introduction

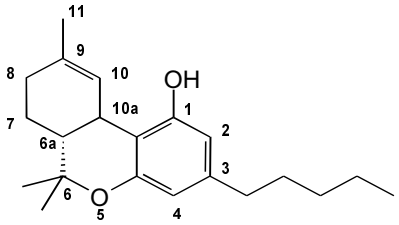
Cannabis sativa is the unique source of more than sixty oxygen-containing aromatic hydrocarbon compounds called cannabinoids. The most active member of this family is Δ^9-tetrahydrocannabinol (Δ^9-THC) to which most of the known pharmacologic properties of cannabis are attributed. Δ^9-THC exerts its most prominent effects on the central nervous system (CNS) [1, 2] and on the cardiovascular system [3–5]. It is well known that cannabinoids also have intraocular pressure (IOP) lowering effects [6–8]. Thus, they have a therapeutic potential in the treatment of glaucoma – IOP being one of many factors associated with the development and progression of this disease. It was found that the degree of IOP-lowering effects of Δ^9-THC and other cannabinoids is at least as great as with conventional eye drops, such as pilocarpine, and the duration of effect is often longer [9]. Due to the considerable interest related to its actions, the cannabinoid field is thoroughly investigated. It is now known that the main cannabinoid effects are mediated by specific cannabinoid receptors CB1 and CB2; CB1 receptors being present in the central nervous system and in certain neuronal and non-neuronal tissues, and CB2 receptors being present mainly in the cells of immune system [10]. Various cannabinoid ligands and antagonist were identified, and the structure-activity relationship (SAR) of classical cannabinoid ligands is well defined. Accordingly, the benzopyran ring, the phenolic group, a side chain in position 10a, and a bulky side chain in position 3 seem to be requirements for activity and receptor recognition [8, 11–13].

2. Investigations, results, and discussion

2.1. Design

Considering the potential of cannabinoids in lowering IOP we aimed to develop a safe anti-glaucoma agent by separating the desired IOP lowering effect of cannabinoids from their unwanted CNS and cardiovascular side effects using a rational soft drug design approach [14–18]. A number of soft drugs designed by a rational integration of metabolic considerations into the drug design process already proved successful in a variety of fields, such as soft anticholinergics, soft β-blockers, or soft corticosteroids.

Among various soft drug design strategies, the "inactive metabolite-based" and the "soft analogue" approaches are the most useful for designing safe and selective drugs. In this study both approaches were integrated. First, we chose a lead compound, which is a known biologically active compound. Then, a metabolically vulnerable moiety



Δ^9-THC

that provides an exposed spot for enzymatic attack is built into the structure of this lead compound. This vulnerable moiety is preferentially an ester group, which is located so that the overall physical, physicochemical, steric, and complementary properties of the new soft compound are very close to those of the lead compound. In a generally applicable isosteric/isoelectronic type soft analogue design, an ester or a reversed ester moiety replaces neighboring methylene groups. The resulting topically applied soft drug is readily metabolized into a more hydrophilic and inactive molecule that is rapidly excreted (Fig. 1). Hence, the desired activities will be produced virtually exclusively at the target site or near the place of application, as the deactivation takes places everywhere in the body. The metabolism takes place as soon as the desired role is achieved not allowing other type of metabolic routes. As a result of their rational design, the soft drugs are expected to have topical effect, but no systemic one.

In the present design of a soft anti-glaucoma agent, we chose as lead compound **11**, a nitrogen containing cannabinoid analogue that was shown to have IOP lowering activity [19]. A full library of soft analogues was gener-

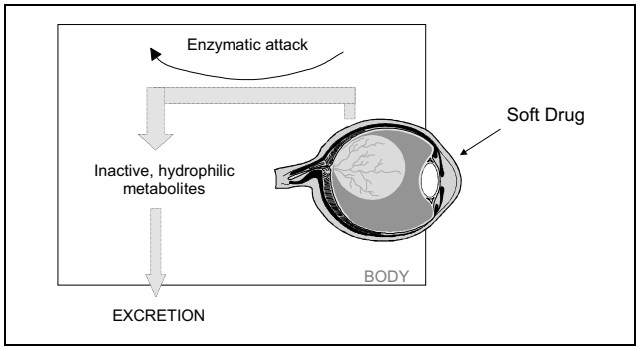


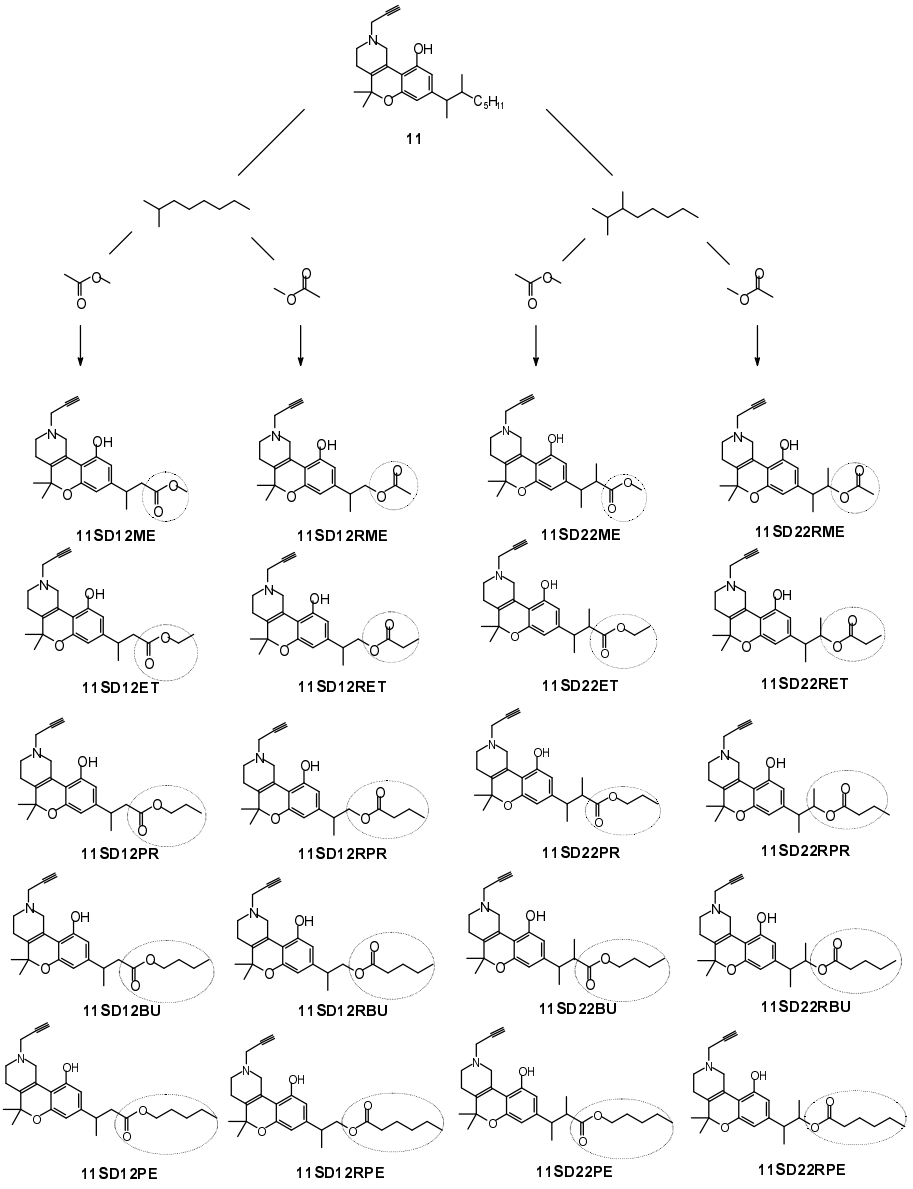
Fig. 1: Separation of systemic and local effects for soft drugs administered in the eye. After exerting its desired therapeutic effect, the topically applied soft drug is readily metabolized into a more hydrophilic and inactive molecule that is rapidly excreted. As a result of its rational design, the soft drug has topical effect, but no systemic one

ated by an expert system developed in our laboratories [20, 21]. The generated soft agents have esters or reverse esters inserted in the long side chain of the lead (Scheme 1). During hydrolysis, the soft drugs will lose a considerable portion of their side chain, and, hence, the formed metabolites are expected to have no cannabinoid activity.

2.2. Ranking

The compounds generated were ranked based on the closeness of calculated properties to those of the lead compound **11**. The hypothesis used is that since the lead is a known active agent, the analogue structures that provide the closest isosteric/isoelectronic analogy will also be more promising drug candidates. Four parameter categories were used with equal weights to describe isosteric/isoelectronic relations: molecular size/shape characterized by volume, surface, and ovality; electric/electronic properties defined by dipole moment, polarizability, and ionization potential; solubility/partition described by calculated log *P* and log *W*; and atomic charge distribution. The data used to describe these properties can be found in the output following AM1 [22] calculations. Values obtained for the lead compound are: *V*: 408.58 Å^3, *S*: 493.69 Å^2, *O*: 1.85, *D*: 0.99 D, α: 35.44 Å^3, *I*: 8.36 eV, BLOGP: 1.56, BLOGW: 3.49. These properties are measured in different units and vary over different ranges; therefore, ranking factors (RF) had to be introduced to be able to compare them. For each property, differences compared to the lead were computed. The ranking factor for the compound with the smallest difference was considered as unity, and for all other compounds the relative values were calculated as RFs. Within each category, the averages of the RFs were computed. The final value ($RF_{AVERAGE}$) is the average of RFs for the four-parameter categories (Table). Smaller numbers indicate better isosteric/isoelectronic analogy. Depending on structure, the estimated *in vitro* human blood hydrolysis half-lives [23] of different soft analogues varied over a wide range (1–1892 min) indicating that various metabolic half-lives can be achieved by structural modifications.

Scheme 1



Soft drugs generated using the nitrogen-containing cannalinoid analog **11** as lead compound

Table: Ranking order of soft analogues also including compound 12 obtained as a byproduct during synthesis of the lead

Filename	Formula	RF_{Size}	$RF_{D/P/Ion}$	$RF_{Sol/Part}$	RF_{Char}	$RF_{Average}$
11SD23ET	$C_{25}H_{33}N_1O_4$	4.51	2.59	13.30	1.53	5.48
11SD24RME	$C_{25}H_{33}N_1O_4$	4.41	1.57	15.16	1.16	5.58
11SD24RPR	$C_{25}H_{33}N_1O_4$	2.49	11.54	10.88	1.85	6.69
11SD22PR	$C_{25}H_{33}N_1O_4$	2.84	9.70	12.21	2.20	6.74
11SD23RET	$C_{25}H_{33}N_1O_4$	4.04	9.84	12.39	1.14	6.85
11SD15RME	$C_{25}H_{33}N_1O_4$	2.60	2.42	11.25	11.14	6.85
11SD24ME	$C_{25}H_{33}N_1O_4$	6.23	4.93	16.73	1.00	7.22
11SD15ME	$C_{25}H_{33}N_1O_4$	2.40	6.40	9.02	11.24	7.26
11SD14RET	$C_{25}H_{33}N_1O_4$	2.39	7.26	10.52	11.46	7.91
11SD14ET	$C_{25}H_{33}N_1O_4$	2.98	4.54	18.79	11.57	9.47
11SD12RPR	$C_{24}H_{31}N_1O_4$	11.41	45.56	14.94	2.84	18.69
11SD13RE	$C_{24}H_{31}N_1O_4$	11.71	53.49	13.46	1.60	20.06
11SD12PR	$C_{24}H_{31}N_1O_4$	11.52	52.92	15.39	2.80	20.66
12	$C_{26}H_{39}N_1O_2$	1.88	66.48	7.75	10.48	21.65
11SD14RME	$C_{24}H_{31}N_1O_4$	13.63	54.38	19.45	1.78	22.31
11SD11BU	$C_{24}H_{31}N_1O_4$	14.44	47.21	22.32	5.27	22.31
11SD14ME	$C_{24}H_{31}N_1O_4$	13.78	60.80	18.20	2.13	23.73
11SD11RBU	$C_{24}H_{31}N_1O_4$	13.47	43.79	18.32	20.22	23.95
11SD13ET	$C_{24}H_{31}N_1O_4$	14.81	57.65	21.59	2.79	24.21
11SD22RET	$C_{24}H_{31}N_1O_4$	14.13	57.75	20.37	6.90	24.79
11SD22ET	$C_{24}H_{31}N_1O_4$	15.62	58.13	23.94	2.09	24.95
11SD23RME	$C_{24}H_{31}N_1O_4$	16.61	59.25	23.30	1.17	25.08
11SD2NOCO	$C_{25}H_{33}N_1O_4$	6.43	39.56	12.80	46.36	26.29
11SD16ME	$C_{26}H_{35}N_1O_4$	13.51	56.20	35.52	1.63	26.71
11SD11OME	$C_{26}H_{35}N_1O_4$	13.87	53.04	36.39	4.62	26.98
11SD24RET	$C_{26}H_{35}N_1O_4$	6.78	68.91	27.68	6.94	27.58
11SD25ME	$C_{26}H_{35}N_1O_4$	5.45	56.44	43.46	6.63	27.99
11SD23ME	$C_{24}H_{31}N_1O_4$	17.47	70.72	26.56	1.50	29.07
11SD24ET	$C_{26}H_{35}N_1O_4$	7.42	67.44	35.19	7.07	29.28
11SD2NCO	$C_{25}H_{33}N_1O_4$	6.58	19.10	51.08	41.72	29.62
11SD1NOCO	$C_{24}H_{31}N_1O_4$	10.87	57.55	4.60	46.93	29.99
11SD15ET	$C_{26}H_{35}N_1O_4$	13.35	64.56	35.78	12.10	31.45
11SD25RME	$C_{26}H_{35}N_1O_4$	4.93	71.53	43.12	6.70	31.57
11SD1OCME	$C_{26}H_{35}N_1O_4$	8.62	74.76	37.54	7.73	32.16
11SD15RET	$C_{26}H_{35}N_1O_4$	13.28	68.40	37.32	11.44	32.61
11SD1OCME	$C_{26}H_{35}N_1O_4$	10.44	85.99	39.50	7.02	35.74
11SD1NCO	$C_{24}H_{31}N_1O_4$	8.81	58.41	39.98	42.19	37.35
11SD12RET	$C_{23}H_{29}N_1O_4$	24.94	109.68	30.25	2.21	41.77
11SD13RME	$C_{23}H_{29}N_1O_4$	24.95	119.10	26.31	1.65	43.00
11SD12ET	$C_{23}H_{29}N_1O_4$	23.55	117.47	30.00	2.43	43.36
11SD2O2ME	$C_{27}H_{37}N_1O_4$	19.66	93.97	56.61	3.28	43.38
11SD11PR	$C_{23}H_{29}N_1O_4$	27.56	111.05	33.60	5.29	44.38
11SD11RPR	$C_{23}H_{29}N_1O_4$	27.23	105.94	30.56	20.26	46.00
11SD13ME	$C_{23}H_{29}N_1O_4$	25.95	127.14	32.66	1.63	46.84
11SD22RME	$C_{23}H_{29}N_1O_4$	27.19	123.93	30.91	6.95	47.25
11SD22ME	$C_{23}H_{29}N_1O_4$	29.00	126.27	36.25	2.16	48.42
11SD2O1ME	$C_{27}H_{37}N_1O_4$	19.67	114.13	59.19	4.27	49.32
11SD26ME	$C_{27}H_{37}N_1O_4$	20.29	117.15	62.04	1.25	50.18
11SD1OET	$C_{27}H_{37}N_1O_4$	23.65	123.00	59.37	3.00	52.25
11SD16ET	$C_{27}H_{37}N_1O_4$	23.51	125.92	61.38	2.37	53.30
11SD2OCME	$C_{27}H_{37}N_1O_4$	16.81	127.42	62.05	7.45	53.43
11SD25ET	$C_{27}H_{37}N_1O_4$	18.00	123.77	71.36	6.62	54.94
11SD2OCME	$C_{27}H_{37}N_1O_4$	15.53	145.25	59.66	6.91	56.84
11SD25RET	$C_{27}H_{37}N_1O_4$	16.82	133.77	72.58	6.79	57.49
11SD1OCET	$C_{27}H_{37}N_1O_4$	23.32	133.89	68.27	7.67	58.29
11SD1OCET	$C_{27}H_{37}N_1O_4$	19.96	144.07	64.10	7.69	58.95
11SD12RME	$C_{22}H_{27}N_1O_4$	38.23	175.65	45.08	2.25	65.30
11SD11ET	$C_{22}H_{27}N_1O_4$	39.96	174.32	48.57	4.86	66.93
11SD12ME	$C_{22}H_{27}N_1O_4$	37.27	185.78	46.34	2.48	67.97
11SD11RET	$C_{22}H_{27}N_1O_4$	40.48	169.54	41.83	20.20	68.01
11SD2O2ET	$C_{28}H_{39}N_1O_4$	33.54	154.39	89.00	3.45	70.10
11SD2O1ET	$C_{28}H_{39}N_1O_4$	32.70	180.25	91.12	4.40	77.12
11SD26ET	$C_{28}H_{39}N_1O_4$	33.14	184.42	95.41	1.85	78.71
11SD2OCET	$C_{28}H_{39}N_1O_4$	30.21	187.14	94.41	7.33	79.77
11SD2OCET	$C_{28}H_{39}N_1O_4$	28.23	208.20	91.83	5.96	83.55
11SD11RME	$C_{21}H_{25}N_1O_4$	53.94	235.50	53.41	20.26	90.78
11SD11ME	$C_{21}H_{25}N_1O_4$	54.89	245.12	61.18	5.45	91.66

The four parameter-categories used with equal weights for ranking are solubility/partition, molecular size/shape, electric/electronic properties, and atomic charge distribution. The final value is the average of the ranking factors (RFs) of the four parameter-categories. The smaller the final value, the better is the analogy between lead and analogue

2.3. Chemistry

The alcohol **3** was prepared according to the literature [24] (Scheme 2). Its direct hydrogenation was very slow as reported, but addition of TFA led to the desired alkane. Interestingly, dehydration with $POCl_3$/pyridine gave essentially pure exocyclic alkene, which was easily hydrogenated. The alkane (**5**) consists of two diastereomers, direct hydrogenation gave a 7 : 3.2 ratio; hydrogenation of alkene (**4**) gave a 2.3 : 1.8 ratio. Demethylation and condensation with piperidine (**7**) gave the coumarin **8** whose structure was confirmed by ^1H and ^{13}C NMR.

We were unable to prepare the *N*-benzylpyran **9** by Grignard reaction as described by Pars et al. [24]. Preparation failed during the work-up; thus, a new work-up procedure with two consecutive steps was followed which gave the desired product (Scheme 3). The *N*-benzylpyran **9** was catalytically debenzylated over palladium-charcoal and then alkylated with propargyl bromide to yield the final compound **11** (Scheme 4) [24]. The alkylation gave the final product **11** and a structurally similar byproduct **12**; the latter most probably gained from the debenzylation by hydrogenation of the double bond.

Another synthetic pathway was attempted for the preparation of the necessary intermediate alcohol **3** to achieve a better yield. The starting material, 3,5-dihydroxyacetophenone, was first methylated with dimethylsulfate and with methyl iodide. The latest gave a better yield. Methylation was followed by Grignard reaction. This attempt unfortunately was unsuccessful due to enolization of the ketone with the corresponding Grignard reagent. Another attempt using 3,5-dibenzyloxyacetophenon as starting material was unsuccessful as well due to enolization of the ketone used.

2.4. Pharmacological effects

The *in vivo* effects on IOP of **11** and **12** were evaluated in a preliminary study in rabbits. The obtained results indicate that despite some solubility problems, IOP lowering

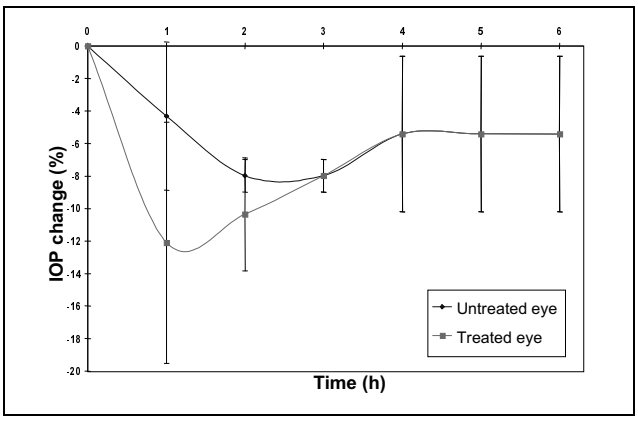
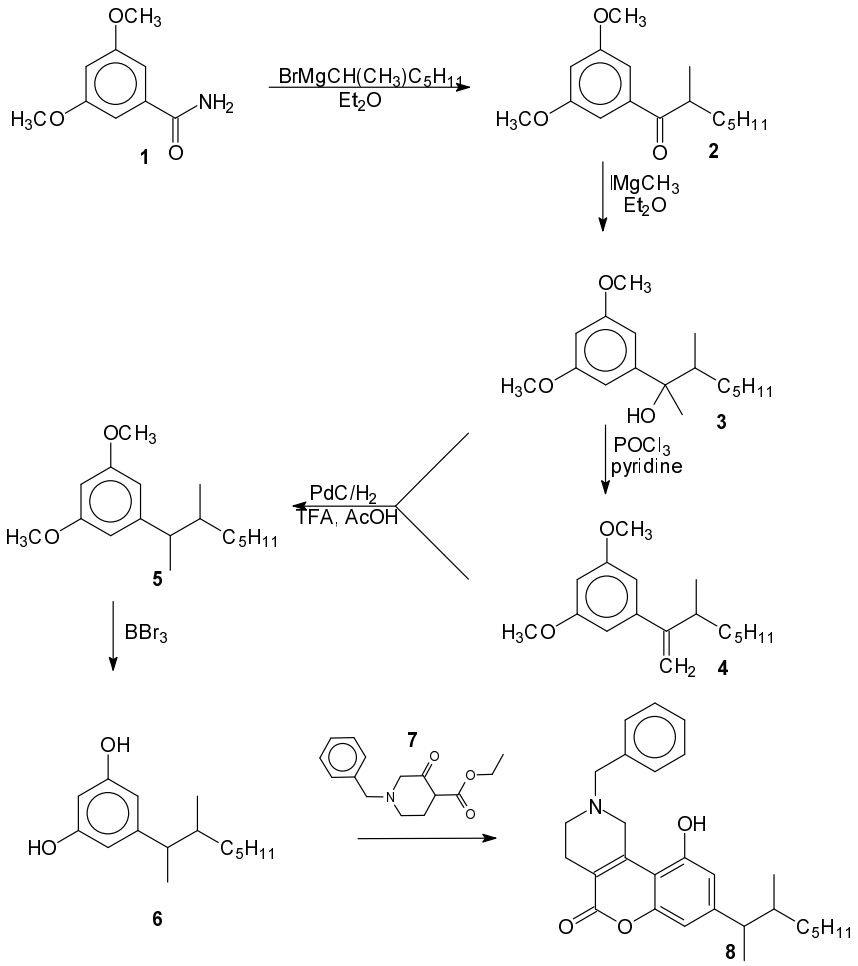


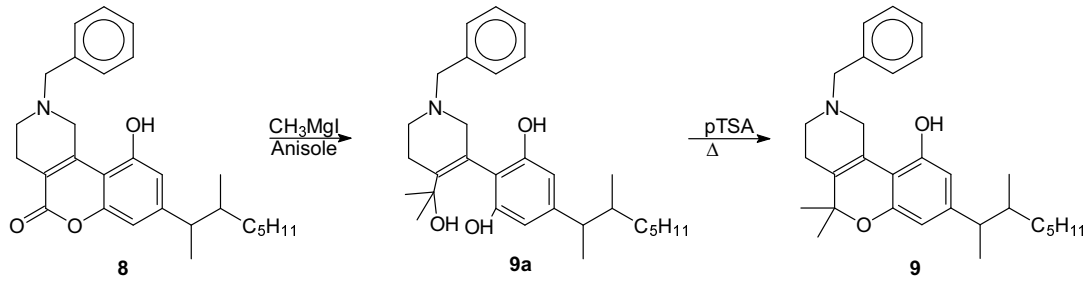
Fig. 2: Percent drop of IOP in rabbits following administration of compound 11 (1% light mineral oil solution) in one of the eyes. The other eye served as control. Data represents mean ± SD of three rabbits

Scheme 2



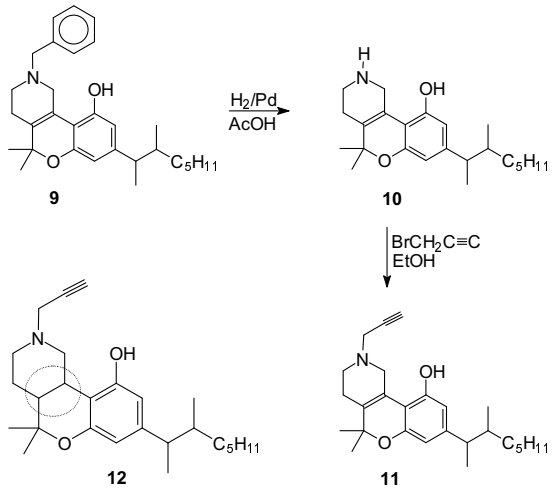
Preparation of the substituted resorcinol **6** followed by Pechmann reaction with ethyl 1-benzyl-3-oxo-4-piperidinecarboxylate hydrochloride (**7**) to give the desired *N*-benzylpyrone **8** [25]

Scheme 3



The new work-up procedure with two consecutive steps that gave the desired product *N*-benzylpyran **9**

Scheme 4



Synthesis of the lead compound **11**. The Grignard reaction gives the *N*-benzylpyran **9**, which is catalytically debenzylated to compound **10** and then alkylated with propargyl bromide to yield the final compound **11** [24]. A similar byproduct **12** was obtained during synthesis

activity is present in the lead compound (Fig. 2). Because of the isosteric/isoelectronic analogy, we expect this activity to be also present in the soft analogues whose metabolic liability will ensure the lack of undesired side effects. Because of the high variation of the data, a definitive result cannot be concluded for compound **12**. However, its predicted effect is relatively consistent with the results obtained. The structure of **12** was included in the ranking done by the same expert system developed in our laboratories based on the closeness of their physical-chemical properties to that of the lead compound **11** as shown in the Table.

The structure-activity relationship and preliminary biological experiments, thus, indicate a good possibility for the development of a safe and soft anti-glaucoma agent. The synthetic pathways of the best soft candidates predicted to have the closest isosteric/isoelectronic properties to that of the lead compound and the pharmacological activity testings are currently under investigation.

3. Experimental

3.1. Chemistry

Melting points were taken on a Fisher-Jones apparatus and are uncorrected. All synthesized products were characterized by FAB (fast atom bombardment) or ESI-MS (electrospray ionization) mass spectrometer. ^{1}H NMR spectra were recorded on a 300 MHz instrument. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). TLC was carried out on silica gel coated glass (Whatman MK6F 250 μm thickness) or on silica-gel coated aluminum plates (Kieselgel 60 F_{254}, 0.2 mm thickness). Column chromatography was performed using silica gel. Distillation was performed with Aldrich Kugelrohr ball-tube distillation apparatus or short-path distillation apparatus.

3.1.1. [3,5-Dimethoxyphenyl 1-methylhexyl ketone] (**2**)

Ketone **2** was synthesized following the general method of Suter and Weston for the synthesis of 3,5-dimethoxyphenyl alkyl ketones. The product, b.p. 130 °C (0.2 mm Hg), was identified by ^{1}H NMR ($CDCl_3$) δ: 0.84–0.91 (3 H, m, $CH_2C\underline{H}_3$), 1.18 (3 H, d, $CHC\underline{H}_3$), 1.22–1.34 (8 H, br, $(CH_2)_4$), 3.34–3.45 (1 H, m, CH), 3.84 (6 H, s, OCH_3), 6.65 (1 H, t, $OCH_3\underline{Ph}OCH_3$), 7.09 (2 H, d, $\underline{Ph}C{=}O$) ppm. The yield was 93%. Literature data [25] for this compound: b.p. 147 °C (1.0 mm Hg) with yield of 82%.

3.1.2. [3-Methyl-2-(3,5-dimethoxyphenyl)-2-octanol] (**3**)

172 mmol (45.5 g) of ketone **2** dissolved in 100 ml ether was mixed with 172 mmol (68 ml) of methyl magnesium iodine. After stirring overnight in argon atmosphere, 250 ml of saturated aqueous solution of ammonium chloride was added dropwise with stirring. The ethereal layer was separated, washed with aqueous sodium bicarbonate and water, and dried over sodium sulfate. After purification, 44.99 g of the tertiary alcohol **3** was obtained. The product was identified by ^{1}H NMR ($CDCl_3$) δ: 0.74–0.82 (3 H, t, $CH_2C\underline{H}_3$), 1.13–1.18 (8 H, br, $(CH_2)_4$), 1.39 (3 H, s, CCH_3), 1.42 (3 H, d, $CHC\underline{H}_3$), 1.66 (1 H, m, CH), 3.72 (6 H, s, OCH_3), 5.22 (1 H, s, OH), 6.27 (1 H, t, $OCH_3\underline{Ph}OCH_3$), 6.51 (2 H, d, $\underline{Ph}COH$) ppm. The yield was 93%.

3.1.3. [3-Methyl-2-(3,5-dimethoxyphenyl)-octene] (**4**)

A solution of 73 mmol (20.46 g) of the tertiary alcohol **3** with 100 ml pyridine was added to 272 mmol (25 ml) phosphorus oxychloride. After the addition, the ice bath was taken away, and the mixture was stirred overnight in argon atmosphere. Work-up was done with 5% aqueous sulfuric acid solution. The organic methylene chloride layer was washed with aqueous sodium bicarbonate and water, and dried over sodium sulfate. The product was distilled to give 17.91 g of product **4**, b.p. 110 °C (0.6 mm Hg), which was identified by ^{1}H NMR ($CDCl_3$) δ: 0.84–0.88 (3 H, m, $CH_2C\underline{H}_3$), 1.10 (3 H, d, $CHC\underline{H}_3$), 1.22–1.31 (8 H, br, $(CH_2)_4$), 2.54–2.65 (1 H, m, CH), 3.80 (6 H, s, OCH_3), 5.00, 5.17 (2 H, d, $C{=}CH_2$), 6.38 (1 H, t, $OCH_3\underline{Ph}OCH_3$), 6.49 (2 H, d, $\underline{Ph}C{=}C$) ppm. The yield was 93%. Literature data [25] for this compound was: b.p. 132–134 °C (1.0 mm Hg) with yield of 85%.

3.1.4. [3-Methyl-2-(3,5-dimethoxyphenyl)-octane] (**5**)

A solution of 102 mmol (26.7 g) of **4** with 140 ml glacial acetic acid was mixed with a 10% solution of palladium on activated carbon and glacial acetic acid, and the mixture was shaken under hydrogen. After 4 h, the mixture was filtrated; the filtrate was evaporated, and the residue was dissolved in ether and neutralized with aqueous sodium bicarbonate. The ether layer was washed with water, dried over sodium sulfate, and then the solvent was evaporated. Distillation gave 49.76 g of the product **5** b.p. 130 °C (0.6 mm Hg), which was identified by ^{1}H NMR ($CDCl_3$) δ: 0.75 (3 H, d, $CHCH(C\underline{H}_3)CH_2$), 0.8–0.92 (3 H, m, $CH_2C\underline{H}_3$), 1.21 (3 H, d, $PhCHC\underline{H}_3$), 1.14–1.34 (8 H, br, $(CH_2)_4$), 1.54–1.66 (1 H, m, $CHC\underline{H}(CH_3)CH_2$) 2.42–2.55 (1 H, m, $\underline{CH}Ph$), 3.77 (6 H, s, OCH_3), 6.30 (1 H, t, $OCH_3\underline{Ph}OCH_3$), 6.32, 6.33 (2 H, 2t, $\underline{Ph}CH$) ppm. The yield was 92%. Literature data [25] for this compound was: b.p. 120 °C (0.5 mm Hg) with yield of 79%.

3.1.5. [3-Methyl -2-(3,5-dihydroxyphenyl)-octane] (**6**)

94 mmol (24.88 g) of compound **5** dissolved in 100 ml of methylene chloride was added to a solution of 100 ml boron tribromide in 40 ml of methylene chloride after cooling with dry ice and acetone. The mixture was cooled continuously for 1 h and stirred overnight in argon atmosphere. In

the reaction mixture, ice water and an equivalent amount of ether was poured. After the organic layer was separated, 2.5% of sodium hydroxide solution was added, then the aqueous layer was washed with ether and acidified with concentrated hydrochloric acid. The organic layer was extracted two times and dried over sodium sulfate. Distillation gave 21.3 g of material boiling at aprox. 130–140 °C at 0.2 mm Hg pressure. The product **6** was identified by ^{1}H NMR ($CDCl_3$) δ: 0.74 (3 H, d, $CHCH(C\underline{H}_3)CH_2$), 0.81–0.92 (3 H, m, $CH_2C\underline{H}_3$), 1.18 (3 H, d, $PhCHC\underline{H}_3$), 1.12–1.34 (8 H, br, $(CH_2)_4$), 1.50–1.62 (1 H, m, $CHC\underline{H}(CH_3)CH_2$), 2.36–2.50 (1 H, m, $C\underline{H}Ph$), 4.70 (2 H, s, OH), 6.17 (1 H, t, $OH\underline{Ph}OH$), 6.26 (2 H, t, $\underline{Ph}CH$) ppm. The yield was 96%. Literature data [25] for this compound: b.p. 167–169 °C (1.0 mm Hg) with yield of 80%.

3.1.6. [2-Benzyl-8-(1,2-dimethylheptyl)-10-hydroxy-5-oxo-1,2,3,4-tetrahydro-5 H-[1]benzopyrano[3,4-d]pyridine] (**8**)

The pyrone **8** was synthesized after the method reported by Pars et al. [24].
The product was identified by ^{1}H NMR ($CDCl_3$) δ: 0.59 (3 H, d, $CHCH(C\underline{H}_3)CH_2$), 0.68–0.81 (3 H, m, $CH_2C\underline{H}_3$), 1.04 (3 H, d, $PhCHC\underline{H}_3$), 0.96–1.32 (8 H, br, $(CH_2)_4$), 1.36–1.50 (1 H, m, $CHC\underline{H}(CH_3)CH_2$), 2.28–2.38 (1 H, m, $C\underline{H}C{=}CH$), 2.65 (2 H, t, $C\underline{H}_2CH_2N$), 3.67 (2 H, s, $C{=}CCH_2N$), 4.05 (2 H, s, $PhC\underline{H}_2N$), 6.13 (1 H, s, OH), 6.33 (1 H, s, $OH\underline{Ph}CH$), 6.56 (1 H, s, $OCO\underline{Ph}CH$), 7.18–7.29 (5 H, m, Ph) ppm.

3.1.7. [2-Benzyl-5,5-dimethyl-8-(1,2-dimethylheptyl)-10-hydroxy-1,2,3,4-tetrahydro-5H-[1]benzopyrano[3,4-d]pyridine] (**9**)

The reaction published by Pars et al. [24] was followed, but a new work-up procedure with two consecutive steps was used. The excess Grignard reagent was decomposed with saturated ammonium chloride water solution. The mixture was extracted with approx. equivalent amount of ether, dried over potassium carbonate and filtered. This work-up was followed by recyclization: 2 g of pTSA (*p*-toluenesulfonic acid monohydrate) were added to the filtrate. The mixture was stirred overnight under argon atmosphere at 60 °C. Next day the reaction was crashed with saturated sodium bicarbonate aqueous solution. The organic layer was separated with ethyl ether and dried over sodium sulfate. The ether extract was evaporated. The anisole was distilled off, and the product was triturated by chloroform. The product **9** was identified by ^{1}H NMR ($CDCl_3$) δ: 0.71 (3 H, d, $CHCH(C\underline{H}_3)CH_2$), 0.79–0.89 (3 H, m, $CH_2C\underline{H}_3$), 1.14 (3 H, d, $PhCHC\underline{H}_3$), 1.07–1.30 (8 H, br, $(CH_2)_4$), 1.32 (6 H, s, $(C\underline{H}_3)_2C$), 2.16–2.24 (1 H, m, $CH_2C\underline{H}CH_3$), 2.27–2.39 (1 H, m, $PhC\underline{H}CH_3$), 2.59 (2 H, t, $CH_2C\underline{H}_2N$), 2.76–2.90 (2 H, t, $C\underline{H}_2CH_2N$), 3.59 (2 H, s, $C{=}CC\underline{H}_2N$), 3.65 (2 H, s, $PhC\underline{H}_2N$), 6.15 (1 H, s, $C(CH_3)_2CO\underline{Ph}CH$), 6.16 (1 H, s, $OH\underline{Ph}CH$), 6.17 (1 H, s, OH), 7.20–7.32 (5 H, m, Ph) ppm. The yield was 53%.

3.1.8. [5,5-Dimethyl-8-(1,2-dimethylheptyl)-10-hydroxy-1,2,3,4-tetrahydro-5H-[1]benzopyrano[3,4-d]pyridine] (**10**)

Compound **10** was synthesized after the method reported by Pars et al. [24].
The product was identified by ^{1}H NMR ($CDCl_3$) δ: 0.73 (3 H, d, $CHCH(C\underline{H}_3)CH_2$), 0.81–0.90 (3 H, m, $CH_2C\underline{H}_3$), 1.16 (3 H, d, $PhCHC\underline{H}_3$), 1.10–1.30 (8 H, br, $(CH_2)_4$), 1.33 (6 H, s, $(C\underline{H}_3)_2C$), 2.11–2.20 (1 H, m, $C\underline{H}(CH_3)CH_2$), 2.29–2.42 (1 H, m, $PhC\underline{H}CH_3$), 3.06 (2 H, t, $CH_2C\underline{H}_2N$), 3.78 (2 H, t, $C\underline{H}_2CH_2N$), 3.98 (2 H, s, $C{=}CCH_2N$), 6.07 (1 H, s, $(CH_3)_2CO\underline{Ph}CH$), 6.21 (1 H, s, $OH\underline{Ph}CH$) ppm. The yield was 84%.

3.1.9. [5,5-Dimethyl-8-(1,2-dimethylheptyl)-10-hydroxy-2-(2-propynyl)-1,2,3,4-tetrahydro-5H-[1]benzopyrano[3,4-d]pyridine] (**11**)

Compound **11** was synthesized according to the method reported by Pars et al. [24].
Recrystallization from CH_3CN gave the final product and a structurally similar byproduct **12** (Scheme 4), which were separated by HPLC. The HPLC column used was DuPont Instrument C18; the solvent was a mixture of 85% methanol and 15% water; detection was made at wavelength of 280 nm, and the flow rate was 1 ml/min. The separated compounds were identified by MS: $m/z = 396.32$, $(M + 1)$, $m/z = 398.34$, $(M + 1)$, respectively. Compound **11** was also identified by ^{1}H-NMR (DMSO-d_6) δ: 0.71 (3 H, d, $CHCH(C\underline{H}_3)CH_2$), 0.79–0.89 (3 H, m, $CH_2C\underline{H}_3$), 1.12 (3 H, d, $PhCHC\underline{H}_3$), 1.14–1.22 (8 H, br, $(CH_2)_4$), 1.25 (6 H, s, $(C\underline{H}_3)_2C$), 2.15–2.23 (1 H, m, $C\underline{H}(CH_3)CH_2$), 2.27–2.36 (1 H, m, $PhC\underline{H}CH_3$), 2.53 (2 H, t, $CH_2C\underline{H}_2N$), 2.59 (1 H, t, $CH{\equiv}C$), 2.95 (2 H, t, $C\underline{H}_2CH_2N$), 3.36 (2 H, s, $C{=}CCH_2N$), 3.52 (2 H, s, $HC{\equiv}CC\underline{H}_2N$), 6.07 (1 H, s, $C(CH_3)_2CO\underline{Ph}CH$), 6.19 (1 H, s, $OH\underline{Ph}CH$), 9.25 (1 H, s, OH) ppm.

3.1.10. In vivo IOP testing

Solutions of 1% of **11** and **12** prepared with light mineral oil were administered to three male, normotensive, New Zealand Albino rabbits (3.5–4 kg body weight). The rabbits were kept in individual cages with free access to food and water. A Digilab Model 30R pneumatonometer was used to measure the intraocular pressure (IOP) [26]. Procaine hydrochloride 0.5% was used as local anesthetic. One drop of this solution was administered immediately prior to each measurement. After the control pressure was taken, one drop (50 µl) of drug was administered in one eye, the other eye served as control. The IOPs of both eyes were measured every hour up to six hours. The percent reduction of IOP was calculated as the difference between the IOPs of the treated and the control eyes divided by the control IOP taken before drug application.

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