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Rational Design, Synthesis and Structure–Activity Relationships of Antitumor (*E*)-2-Benzylidene-1-tetralones and (*E*)-2-Benzylidene-1-indanones

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Abstract—Novel substituted 6,7-dimethoxy-1-tetralones and 5,6-dimethoxy-1-indanones have been synthesized and evaluated for their cytotoxicity. Compounds with 3′-lipophilic, 3′,5′-dilipophilic, or 3′,5′-dilipophilic-4′-hydrophilic substituents on (E)-2-benzylidene moiety showed highly cytotoxic effects. The unique structure of **42** possibly matches the pharmacophore features for these cytotoxic compounds.

Unlike Aricept, a 2-alkyl-5,6-dimethoxy-1-indanone and a reversible acetylcholinesterase (AChE) inhibitor as an effective treatment for Alzheimer's disease,[1–3] we found some of substituted (*E*)-2-arylmethylene-6,7-dimethoxy-1-tetralones to act as ceramide stimulants which in turn expressed inhibition of tumor cell growth.[4–7] The reduced forms of these compounds by hydrogenation did not show cytotoxicity. These results prompted us to design and synthesize a series of conformational analogues of (*E*)-2-benzylidene-1-tetralone and -1-indanone to optimize the structure–activity relationships and to develop the more potent and selective antitumor agents. Herein, we report the synthesis, systemic investigation of structure–activity relationships of these classes of compounds, and the discovery of remarkably cytotoxic indanorine (**36**)[8] and indanocine (**42**).[9]

Chemistry

The substituted 1-tetralones, **1**–**21**, and 1-indanones, **22**–**34**, **36**, **41**, **43**, **45**–**47**, **49**–**51**, were synthesized by aldol condensation of substituted 1-tetralone or 1-indanone with the respective benzaldehydes under basic or acidic conditions (Scheme 1). The E-configuration of these compounds was determined by ^{1}H NMR according to the method established by Bayer et al.[10] Compounds **37**–**39** were prepared from reaction of **36** with appropriate reagents. Treatment of **39** with Li_2CO_3 in CH_3OH gave **40**. Reaction of 5,6-dimethoxy-1-indanone with $NaNO_2$ in TFA afforded 5,6-dimethoxy-7-nitro-1-indanone,[11] which was condensed with 3,5-dimethyl-4-hydroxy- or 3,5-dimethyl-4-nitrobenzaldehyde to give **41** or **43**, respectively. Reduction of **34**, **41**, **43**, or **47** with $Na_2S_2O_4$ or zinc dust and 2% AcOH in 1,4-dioxane yielded **35**, **42**, **44**, or **48**, respectively (Scheme 1). Compound **52** was obtained by treatment of **30** with BBr_3 in CH_2Cl_2.

Results and Discussion

Biological evaluation

The preliminary in vitro antitumor activity of compounds **1**–**52** was conducted using a formazan dye (MTT) conversion assay[12] against Jurkat cell line (Table 1). Generally, the most cytotoxic compounds are slightly less sensitive to other cell lines including CEM, L1210, B16, Molt-4, WIL2, WI38, K562, HL-60, H596, H292, MCF-7, A549, and NIH3T3 (data not shown). Among 52 compounds, **1**, **6**, **9**, **10**, **13**–**15**, **20**, **41**, **43** and **49**–**52** did not exhibit cytotoxicity. **8**, **25**, **27**, **34**, **38** and **48** showed slight cytotoxicity. The other compounds demonstrated significant to remarkable cytotoxicity. **16**,

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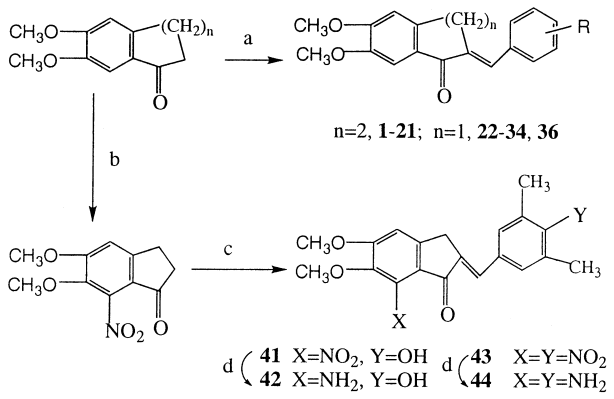


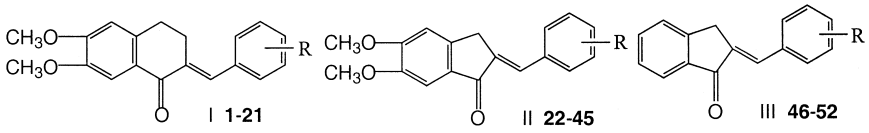
Scheme 1. (a) Substituted benzaldehyde, basic or acidic condition; (b) $NaNO_2$, TFA 0 °C–rt; (c) 3,5-dimethyl-4-hydroxy- or 3,5-dimethyl-4-nitrobenzaldehyde, 5% CH_3SO_3H in AcOH; (d) $Na_2S_2O_4$ or Zn dust and 2% AcOH in 1,4-dioxane, 100 °C.

29–**31**, **36** and **42** were submitted to the National Cancer Institute for testing in vitro cytotoxicity against 60 tumor cell lines.[13,14] Compounds **29**–**31**, **36** and **42** were further screened by NCI for preliminary in vivo testing against tumor cells that were placed in the polyvinylidene fluoride (PVDF) hollow fibers of capsules and then implanted into the intraperitoneal or the subcutaneous compartment in mice.[15] Compounds **29**–**31** did not obtain satisfactory score (IP + SC score < 20). However, **36** and **42** showed very good scores (IP + SC score >20) and underwent further in vivo xenograft studies. Currently, **42** did not show satisfactory results, possibly due to poor bioavailability problems, such as significant protein binding. We are making water soluble derivatives to improve the in vivo profiles. In addition, these compounds appear to act as antimicrotubule agents.[16]

Structure–activity relationships and pharmacophore

According to the Jurkat cell inhibitory effects, the inactive compounds of substituted (E)-2-benzylidene-6,7-dimethoxy-1-tetralones are 2′-lipophilic (**1**), 4′-lipophilic (**6**), 2′,3′- (**9**), 2′,4′- (**10**), 2′,6′- (**13**) and 3′,4′-dilipophilic (**14**), 3′,4′-dihydrophilic (**15**) and 3′,4′,5′-trilipophilic (**20**) substituted derivatives. However, the active compounds are 3′-lipophilic (**2**–**5**), 4′-hydrophilic (**7,8**), 2′,5′- (**11,12**), and 3′,5′-dilipophilic (**16**–**19**) and 3′,5′-dilipophilic-4′-hydrophilic (**21**) substituted derivatives. These results suggest that the lipophilic substituent at the *meta* (3′) position is important for demonstrating cytotoxicity, and both 3′ and 5′ dilipophilic substituents may enhance the biological activity. In addition, the 4′-hydrophilic substituent can provide an additional binding affinity to the binding site in addition to 3′,5′-dilipophilic substituents as seen in **21**. These results lead us to propose a partial pharmacophoric model of 3′,5′-dilipophilic-4′-hydrophilic substituted (*E*)-2-benzylidene moiety of 6,7-dimethoxy-1-tetralone binding to an unidentified binding pocket to demonstrate cytotoxicity.

Table 1. Cytotoxicity of the substituted 1-tetralones and 1-indanones against Jurkat cell growth



Compound	Substituents	IC_{50} μM	Compound	Substituents	IC_{50} μM
1	2′-OCH_3	>50	**27**	2′,5′-di-CH_3	30.5
2	3′-OCH_3	5	**28**	3′,5′-di-F	19
3	3′-Cl	9	**29**	3′,5′-di-OCH_3	3
4	3′-NO_2	9	**30**	3′,5′-di-Cl	2
5	3′-CH_3	17	**31**	3′,5′-di-CH_3	0.08
6	4′-OCH_3	>50	**32**	2′-OH,3′,5′-di-I	2.5
7	4′-OH	19	**33**	3′-CH_3,4′-OH	1.16
8	4′-NH_2	40	**34**	3′,5′-di-CH_3,4′-NO_2	25
9	2′,3′-di-OCH_3	>50	**35**	3′,5′-di-CH_3,4′-NH_2	0.055
10	2′,4′-di-OCH_3	>50	**36**	3′,5′-di-CH_3-4′-OH	0.006
11	2′,5′-di-OCH_3	11	**37**	3′,5′-di-CH_3,4′-$OCOCH_3$	0.4
12	2′,5′-di-CH_3	6	**38**	3′,5′-di-CH_3,4′-$OCOOCH_2CH(CH_3)_2$	21.5
13	2′,6′-di-OCH_3	>50	**39**	3′,5′-di-CH_3,4′-$OCH_2COOC_2H_5$	2.2
14	3′,4′-di-OCH_3	>50	**40**	3′,5′-di-CH_3,4′-OCH_2COOH	6.3
15	3′-COOH-4′-OH	>50	**41**	7-NO_2,3′,5′-diCH_3,4′-OH	>50
16	3′,5′-di-OCH_3	5	**42**	7-NH_2,3′,5′-di-CH_3,4′-OH	0.001
17	3′,5′-di-CH_3	4	**43**	7,4′-di-NO_2,3′,5′-di-CH_3	>50
18	3′,5′-di-Cl	7	**44**	7,4′-di-NH_2,3′,5′-di-CH_3	0.0235
19	3′,5′-di-NO_2	2	**45**	7-OCH_3,3′,5′-di-CH_3,4′-OH	0.125
20	3′,4′,5′-tri-OCH_3	>50	**46**	5-OH,6-OCH_3,3′,5′-di-CH_3,4′-OH	0.53
21	3′,5′-di-CH_3-4′-OH	4.8	**47**	4-NO_2,5-OH,6-OCH_3,3′,5′-di-CH_3,4′-OH	9.3
22	3′-CH_3	1.8	**48**	4-NH_2,5-OH,6-OCH_3,3′,5′-di-CH_3,4′-OH	30
23	3′-Cl-	8	**49**	5-OCH_3,3′,5′-di-CH_3,4′-OH	>50
24	3′-OCH_3	12.8	**50**	6-OCH_3,3′,5′-di-CH_3,4′-OH	>50
25	3′-NO_2	22	**51**	5,6-OCH_2O-,3′,5′-di-CH_3,4′-OH	>50
26	2′-OH,5′-NO_2-	14.5	**52**	5,6-di-OH,3′,5′-di-Cl	>50

Consistent to the cytotoxicity pattern on substituted 1-tetralone analogues, the 1-indanones, **22**–**36** showed slight to remarkable cytotoxicity, especially **31** (IC_{50} 80 nM), **35** (IC_{50} 55 nM) and **36** (Indanorine, IC_{50} 6 nM). **36** is more potent than **35** because the 4′-hydroxy group provides a much stronger binding affinity than the 4′-amino to the refined region of binding protein. The far less active derivatives of **36, 37–40**, suggest that the binding protein has a small and restricted region corresponding to the binding of 3′,5′-dilipophilic-4′-hydrophilic substituents on (*E*)-2-benzylidene moiety. To further examine the structure–activity relationships around the benzene ring of 1-indanone, we found that the large lipophilic 7-nitro group of **41** might cause an incorrect geometry to disfavor the binding to the pocket and resulted in no cytotoxicity. So did the nontoxic **43**. However, **42** expressed remarkable cytotoxicity (IC_{50} 1 nM), more potent than **36** (IC_{50} 6 nM) because the 7-hydrophilic amino group provides an extra docking to the binding protein. Replacing the 4′-hydroxy of **42** with an amino group offered a less active **44** (IC_{50} 23.5 nM), as expected. Replacing the 7-amino of **42** with a 7-methoxy gave a less active **45** (IC_{50} 125 nM) because the methoxy is a nonpolar but weak hydrogen-bond acceptor. This result further provides the evidence that there is a hydrogen binding site in the binding protein around the 7 position and 1-carbonyl of indanone. Demethylation of **36** at the 5 position yielded a less active **46** (IC_{50} 530 nM) because the 5-hydrophilic hydroxy substituent not only forms hydrogen bonding with its neighboring methoxy group but also interferes with the binding affinity of the molecule to the binding site. Introduction of a nitro group at the 4 position of **46** gave a far less cytotoxic **47** (IC_{50} 9.3 μM). Reduction of the nitro group of **47** resulted in a marginally cytotoxic **48** (IC_{50} 30 μM). The weak cytotoxic effects of **47** and **48** suggest that there is no binding site in the binding protein for the 4-substituent of 1-indanone. Instead, the 4-substituent might squeeze or form hydrogen binding with the neighboring 5-hydroxy to intervene in the binding affinity with the protein. The lack of cytotoxicity for monomethoxy compounds, **49** and **50**, suggests that the 5- or 6-substituent of indanone cannot position correct geometry and loses the binding affinity to the hydrophobic region of the pocket. Compound **51** with lipophilic 5,6-dioxymethylene substituents expressed no cytotoxicity because the lipophilic and planar dioxymethylene ring cannot dock to the binding site. Compared to the potent **30** with 5,6-dimethoxy substituents, the inactive **52** with 5,6-dihydroxy substituents provides further evidence that the hydrogen-bond interaction between 5,6-dihydroxy substituted 1-indanone causes the molecule to lose the binding affinity to the protein. In these active compounds, their 5,6-dimethoxy substituents are lipophilic, repulsive and out of the indanone plane. This information indicates that there are steric features in the binding protein around the 5–7 positions of indanone for binding affinity to render cytotoxicity. Furthermore, these results suggest that in addition to hydrophobic and hydrogen binding sites for 3′,5′-dilipophilic and 4′-hydrophilic substituents on (E)-2-benzylidene moiety, the binding pocket crucially contains a hydrophobic region around the 5 and 6 positions and a hydrogen binding site around the 7 position and 1-carbonyl of (E)-2-benzylidene-1-indanone for binding affinity. Since these active compounds share similar conformations and properties to render cytotoxicity, they contain the complementary patterns of ligand atoms, or pharmacophore to form ligand–receptor complex. Thus, indanocine **42** possibly fits the pocket very well and demonstrates remarkable cytotoxicity (Fig. 1). We will take advantage of this model to explore more antitumor agents.

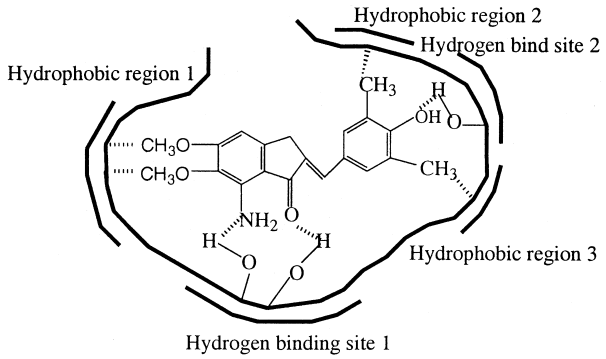


Figure 1. Proposed pharmacophore of indanocine interacting with an unidentified binding pocket.

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9. Mp 238–240 °C; ms 340 (MH^+); ^1H NMR (DMSO-d_6); 2.20 (s, 6H, CH_3), 3.63 (s, 3H, OCH_3), 3.83 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 6.30 (s, 2H, NH_2), 6.50 (s, 1H, C4H), 7.15 (s, 1H, CH), 7.29 (s, 2H, C′2H and C′6H). Anal. for $C_{20}H_{21}NO_4 \cdot 0.2CH_3OH$ C, 70.24; H, 6.35; N, 4.05, found C, 70.24; H, 5.97; N, 3.99.
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