Pharmacophore Mapping of Flavone Derivatives for Aromatase Inhibiiton

Shuchi Nagar, Md Ataul Islam, Arup Mukherjee and Achintya Saha*

Drug Design & Cheminformatics Lab., Div. of Pharmaceutical & Fine Chemical Technology,
Department of Chemical Technology, Univertsity of Calcutta, 92, A.P.C.Road, Kolkata-700009 (India), www.caluniv.ac.in
*email. achintya_saha@yahoo.com

Introduction

*Sex hormones play a crucial physiological role in living organisms and involve in pathophysiological processes, such as tumorgenesis and growth.

*Breast carcinoma is the most common form of female cancer and represents the leading cause of death among women. Brueggemeier R. W. et.al, Anticancer ther., 2002,2,181-191.

*In hormone-dependent breast cancer, estrogen is the proximate regulator of cell proliferation. Brueggemeier R. W. et.al, J.Steroid Biochem Mol Biol, 79, 1-5, 2001, 75-84.

*Two main pharmacological strategies have been employed to control or block the pathological activity of estrogens: Banting L, et.al., Prog Med Chem., 1989,26,253-98

a) Development of drugs that act through the estrogen receptor (ER) antagonists - the selective estrogen receptor modulators (SERMs)

b) Development of drugs that interfere with the synthesis of steroid hormones by inhibiting the enzymes controlling the inter-conversion from androgenic precursors - aromatase inhibitors (AIs).

*SERMs and P450 AIs constitute important therapeutical weapons in the fight against breast cancer deaths. Pasqualini J.R., Biochim Biophys Acta., 2004, 7,1654(2),123-143.

*P450 AIs are currently in clinical use, include the steroidal enzyme inactivator and non-steroidal inhibitor. Miller W.R., Semin. Oncol., 2003, 30, 3-11.

*Steroidal AIs compete with the endogenous ligands, androstenedione and testosterone for the active site of intermediates that bind irreversibly to the active site. Non-steroidal AIs bind reversibly with high affinity to the heme region of the active site. Howell, A., Trends in Endocrin Metabo., 2005,16 (9), 420-428.

*Synthetic AIs increase the risk of coronary artery disease (CAD), vaginal dryness, dysparuenia and loss of interest in sex more frequently. Fallowfield L., et. al., J Clin Oncol. 2004, 22(21), 4261-71.

*Several naturally occurring and synthetic flavonoids - ubiquitous natural phenolic compounds and mediate a host of biological activities are found to demonstrate inhibitory effects on aromatase. Di C.G., et. al., Life Sci. 1999,65(4),337-53, Yeh C.K., et. al., Env. Health Perspective, 1998, 106, 85-92, Jeong H.J. et. al., Arch. Pharm. Res., 1999, 22, 309-312.

*7-methoxyflavonone is a potent AI and also an effective antiproliferative agent against MCF-7 breast cancer cell. Le Bail, J.C., et. al. Cancer Lett. 1998,133(1),101-6; Pouget. C., et. al., Bioorg Med. Chem. Lett., 2001, 11, 3095-3097,

*Flavonoids popularly known as phytoestrogens consumed in substantial amounts from dietary sources, food supplements and more recently as 'nutraceuticals'.

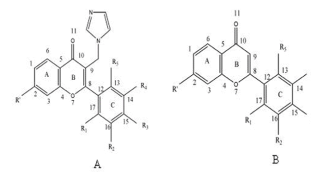
*Aim is to investigate the structural requirements of flavone derivatives for inhibition of aromatase activity with a view to deduce the active pharmacophore signals based on receptor–independent hypothesis.

Materials and Methods

Classical QSAR studies

- QSAR studies are mathematical methodology, statistically validated, and mostly used to correlate experimental or calculated properties derived from chemical structures with biological activities.
- In the present work, 9 compounds belonging to the category of flavone (A) (Gobbi, S., et. al., J. Med. Chem. 2006, 49, 4777-4780) and 24 compounds belonging to flavonone (B) (Pouget, C., et. al., Pharmaceutical Research, 2002, 19(3), 286-291) have been considered (Table 1).
- The P450 19 (CYP19) inhibitory activity (IC_{50}, μM) has been considered as biological activity and implemented as pIC_{50} (log $1000/IC_{50}$).
- Molecular (partition coefficient, hydrophobicity, steric, and thermodynamic factors, bulk, moments and orbital energies), electronic (atomic charge and partial charge functions) and electrotoplogical (E-state indices) properties have been explored for modeling purpose.
- *TSAR 3.3*, *Chem 3D Pro*, *CAChe* and *ETSA-CS* (JAVA based program) are used.
- Statistical analysis is performed by *Statistica 5.0* using standard and forward stepwise multiple regression methods.

Table 1. Structural features and inhibitory activity of flavone(A) and flavonone(B) derivative



Compd no.		R_1	R_2	R_3	R_4	R_5	R'	IC50	pIC50
A	1	H	H	H	H	H	OCH_3	0.55	3.259
	2	H	H	NO_2	H	H	OCH_3	0.47	3.328
	3	H	H	Br	H	H	OCH_3	4.1	2.387
	4	H	H	CN	H	H	OCH_3	1.8	2.744
	5	H	H	H	H	H	H	0.071	4.148
	6	H	H	NO_2	H	H	H	0.045	4.347
	7	H	H	Br	H	H	H	0.44	3.356
	8	H	H	CN	H	H	H	0.069	4.161
	9	H	H	OCH_3	H	H	H	0.080	4.097
B	10	OCH_3	H	H	H	H	OCH_3	6.6	2.180
	11	H	OH	H	H	H	OCH_3	3.5	2.456
	12	H	OCH_3	H	H	H	OCH_3	11.468	1.940
	13	H	H	OH	H	H	OCH_3	3.7	2.432
	14	H	H	OCH_3	H	H	OCH_3	11.415	1.943
	15	H	OH	OH	H	H	OCH_3	2.5	2.602
	16	H	OH	OCH_3	H	H	OCH_3	6.2	2.207
	17	H	OCH_3	OH	H	H	OCH_3	5.4	2.267
	18	H	OH	H	OH	H	OCH_3	3.5	2.456
	19	OCH_3	OCH_3	H	H	H	OCH_3	6.6	2.180
	20	OCH_3	H	OCH_3	H	H	OCH_3	8.0	2.097
	21	OCH_3	H	H	OCH_3	H	OCH_3	19.011	1.721
	22	OCH_3	H	H	H	OCH_3	OCH_3	19.455	1.711
	23	H	OCH_3	OCH_3	H	H	OCH_3	11.71	1.931
	24	H	OCH_3	H	OCH_3	H	OCH_3	15.674	1.805
	25	OCH_3	OCH_3	OCH_3	H	H	OCH_3	4.8	2.318
	26	OCH_3	H	OCH_3	OCH_3	H	OCH_3	17.064	1.768
	27	H	OCH_3	OCH_3	OCH_3	H	OCH_3	14.749	1.831
	28	H	H	Br	H	H	OCH_3	12.136	1.916
	29	H	H	Cl	H	H	OCH_3	10.57	1.976
	30	H	H	F	H	H	OCH_3	11.82	1.927
	31	H	H	CN	H	H	OCH_3	13.054	1.884
	32	H	H	$N(CH_3)$	H	H	OCH_3	30.12	1.521
	33	H	H	H	H	H	OCH_3	8.0	2.097

Pharmacophore space modeling

- A pharmacophore is a set of functional group/fragment types in a spatial arrangement that represents the interaction made in a common scaffold by a set of small molecular ligands with a protein receptor. Kristam R, et al. J Chem Inf Model. 2005, 45(2),461-476.
- Pharmacophore concept is based on the kinds of interaction observed in molecular recognition and alternatively can be used as a query in a 3D database search to identify new structural classes of potential lead compounds.
- The study is performed with this dataset using *CATALYST 4.11*.
- The biological activity is expressed as inhibitory concentration (IC_{50}) to CYP19 inhibition.
- The number of conformer for the compound has been limited to maximum of 250, using 'best conformer generation' with 20 kcal/mol energy cutoff. Spacing used is 300 pm. A uncertainty factor of 2 for each compound has been defined.
- The HypoGen algorithm optimized the chemical features for exploring the spatial pharmacophore map of this group of compounds are hydrogen bond (HB) acceptor (a) and donor (d), hydrophobic (p), and ring aromatic (ra).
- This algorithm is forced to find pharmacophore models that contain at least one and at most two of every features.
- The quality of generated hypotheses are verified through cross-validation technique using Fischer's randomization test, where the biological activity data are randomized within a fixed chemical data set and HypoGen process initiated to explore possibilities of other hypotheses of good predictive values.
- The projected hypothesis is further validated with some reference compounds (standard and non-standard AIs) by measuring fit scores and estimated values of such compounds (Table 5).

Result and Discussion

Classical QSAR studies

The best significant relationship for inhibition of CYP19 has been deduced to be

$$pIC_{50} = 21.906\,(\pm 1.36)\,HC_5 + 2.29\,(\pm\,0.72)\,HC_{12} + 4.48\,(\pm\,1.90)\,O_{11} + 0.51\,(\pm\,0.11)\,\log P - 0.49\,(\pm\,0.12)\,I_{CmOMe} + 8.41\,(\pm\,0.97) \quad \text{Eqn 1}$$

n=33; R=0.972; R2=0.944; EV=93.40%; F=91.6; df=5,27; s=0.0346; AVRES=0.142; PRESS=1.145; SDEP=0.186; Presav =0.0073; Q2=0.943.

- The best uni ($r^2 = 0.86$) and bivariate ($R^2 = 0.88$) relations demonstrated the importance of atom C_5 of chromone ring for CYP19 inhibition.
- The model (Eqn 1) revealed the importance of atoms C_5, O_{11} and C_{12}, and lipophilicity of the molecule for inhibitory activity to CYP19.
- High hydrophobicity is a fundamental physiochemical requirement for CYP19 inhibition. Recanatini, M, J. et al.Med. Chem, 2001, 44, 672-680.
- Atom C_5 is the juncture atom of the chromone ring and the presence of ketonic functional group (O_{11}) at atom C_{10} signified the importance of flavone ring for the activity.
- Presence of bulky substituent at atom C_8 of the flavone scaffold that increases the overall charge function at atom C_{12}, increases the inhibitory activity.
- Negative contribution of the indicator (I_{CmOMe}) that signifies the presence of meta methoxy group at ring C, decreases the activity.
- Flavone scaffold with bulky substituent at atom C_8 that imparts the hydrophobicity of the molecule, increases CYP19 inhibitory activity.

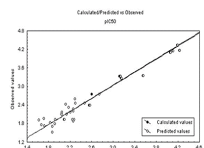


Fig 1: Observed, calculated and predicted values as per Eqn 1

Table 2: Calculated and predicted inhibitory activity to CYP19

Compd no.	pIC_{50} Obs.	Cal.[a]	Pred.[a]	Compd no.	pIC_{50} Obs.	Cal.[a]	Pred.[a]
1	3.260	3.147	3.156	18	2.456	2.468	2.469
2	3.328	3.112	3.133	19	2.180	2.199	2.199
3	2.387	2.553	2.568	20	2.097	2.035	2.041
4	2.745	2.596	2.736	21	1.721	1.722	1.722
5	4.149	4.103	4.111	22	1.711	1.866	1.866
6	4.347	4.186	4.194	23	1.931	2.239	2.240
7	3.356	3.552	3.559	24	1.805	1.910	1.911
8	4.161	4.226	4.235	25	2.319	2.167	2.168
9	4.097	4.057	4.066	26	1.768	1.666	1.666
10	2.180	2.045	2.045	27	1.831	1.826	1.826
11	2.456	2.296	2.296	28	1.916	1.618	1.619
12	1.940	2.272	2.272	29	1.976	1.713	1.713
13	2.432	2.117	2.118	30	1.927	1.918	1.918
14	1.943	2.089	2.090	31	1.884	2.258	2.259
15	2.602	2.285	2.286	32	1.521	1.869	1.870
16	2.208	2.255	2.255	33	2.097	2.133	2.133
17	2.268	2.260	2.260				

[a]as per Eqn 1

Space Modeling Studies

- The proposed hypothesis is characterized on the basis of highest cost difference (Δ), root mean square deviation (rmsd) and the best correlation (R).
- The key pharmacophores of flavone derivatives for inhibition of CYP19 may be deduced to be comprised of HB acceptor (keto functional group), hydrophobic (chromone ring) and ring aromatic features (Fig 2).
- Estimated fit score are delineated in Fig 3.
- The results demonstrated good predictive ability of the model.
- The cross-validation analyses (Fischer's randomization test at 99%) indicate the superiority of the hypotheses considered.
- The critical inter features distances (Table 4) are crucial for imparting the activity.

Table 4: Interfeature distance (Å) of the pharmacophore hypothesis

Features	a	p	ra1	ra2
a	0.000	7.394	4.119	7.938
p		0.000	9.259	8.070
ra1			0.000	5.474
ra2				0.000

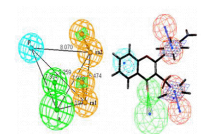
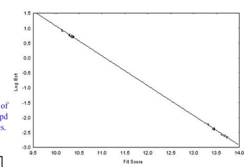


Fig 2: Pharmacophore features of flavonoids for inhibitory activity to CYP19 (hypothesis 1 of run no 4). Mapped features are HB acceptor(a), hydrophobic (p) and ring aromatic (ra). Compd no 6 (most active compound in the training set) is fitted in the mapped pharmacophore features.

Table 3. Hypothesis parameters observed in successive runs.

Run No	Input Features	Uncertainty	Weight variance	Hypothesis No.	Pharmacophore features in generated hypothesis	Cost Null	Cost Fixed	Cost Total	Cost Δcost	R	rmsd
1	a, d, p, ra	3	0.302	1	a, 2 x ra	155.110	125.069	131.113	30.041	0.929	0.603
2	a, ra, pi	2	0.302	1	a, 2 x ra	206.613	109.871	123.348	96.742	0.939	0.886
3*	a, p, ra	2	0.302	1	a, p, 2 x ra	206.613	108.740	122.161	97.873	0.939	0.887
4	a, p, ra	2	2.000	1	a, p, 2 x ra	206.613	109.650	122.454	96.963	0.941	0.876
5#	a, p, ra	2	0.302	1	2 x a, p, ra	206.613	108.612	124.868	98.001	0.924	0.990
6#	a, p, ra	2	0.302	1	2 x a, p, ra	206.613	109.557	125.746	97.056	0.924	0.990
7Ɣ	a, p, ra	2	2.000	1	a, p, ra	206.613	107.610	121.704	99.003	0.934	0.923

a: HB acceptor, d: HB donor; p: Hydrophobic factor, ra: Ring aromatic; *all features, min=1 and max = 2; #for ring aromatic, min = 1 and max = 1; Ɣall input features, min=0 and max = 1; cost = Null cost – Total cost; rmsd = rms deviation;



$r^2 = 1.000$; s = 0.003; n = 33

Fig 3: Estimated activity vs Fit score of the training set

Table 5: Fit score and estimated activity of some reference compounds.

Compd no	Compd name	Fit score	Estimated (μM)	Compd no	Compd name	Fit score	Estimated (μM)
1	Asigenin	8.580	3.1×10^{2}	14	Letrozole	9.029	1.1×10^{2}
2	Cyclofenil	6.830	1.7×10^{4}	15	Nafoxidine	10.782	19
3	Em800*	12.280	6.1×10^{-2}	16	Norgestrel	7.019	1.1×10^{4}
4	Ephedrine	6.940	1.4×10^{4}	17	Qurecetin	8.995	1.2×10^{2}
5	E2	7.010	1.1×10^{4}	18	Stanozolol	6.980	1.2×10^{4}
6	Gefitinib	10.608	2.9	19	Simvastatin	7.019	1.1×10^{4}
7	Genistein	8.440	4.2×10^{2}	20	Tangeretin	9.920	14
8	Hydroxyflavone	9.835	17	21	Tetrahydroisoqunoline	12.727	2.2×10^{-2}
9	Hydroxytamoxifene	9.920	1.3×10^{2}	22	Testosterone	7.018	1.1×10^{4}
10	Hydroxytrioxiene	13.085	9.6×10^{-3}	23	Toremifene	12.735	2.2×10^{-2}
11	Hydroxytoremifene	9.970	13	24	Trioxifene	12.988	1.23×10^{-2}
12	Idoxifene	12.617	2.8×10^{-2}	25	Tse-424**	13.534	3.4×10^{-3}
13	Lasofoxifene	12.740	2.1×10^{-2}				

*Gauthier, S., J. Med. Chem. 1997, 40, 2117-2122, **Miller, C.P., J. Med. Chem. 2001, 44, 1654-1657.

Conclusion

- Aromatic substituents (imidazole and/or phenyl rings) that impart the overall increment of hydrophobicity of the flavonoid derivatives, increase CYP19 aromatase inhibitory activity.
- Keto functional group at atom C_{10} of chromone scaffold behaves as a potential HB-acceptor that might bind with Ser478 of the biological counterpart (Favia, A. D., et al. Proteins, 2006, 62(4), 1074-1087) and produce inactivation of the enzyme.
- The space modeling study correlate with the classical approach of QSAR studies - chromone ring showed the importance of hydrophobic feature of the molecule and the presence of HB-acceptor (keto-functional group) in chromone ring along with aromatic ring features are found to be detrimental for CYP19 inhibition.

Acknowledgement

Financial support from DST FAST Track scheme is thankfully acknowledged.