Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 22, pp. 2903-2908, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10110-X

1-OXO-3-ARYL-1*H*-INDENE-2-CARBOXYLIC ACID DERIVATIVES AS SELECTIVE INHIBITORS OF FIBROBLAST GROWTH FACTOR RECEPTOR-1 TYROSINE KINASE

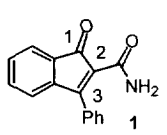
M. R. Barvian,* R. L. Panek, G. H. Lu, A. J. Kraker, A. Amar, B. Hartl, J. M. Hamby, and H. D. H. Showalter

Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co.,
2800 Plymouth Road, Ann Arbor, MI 48105

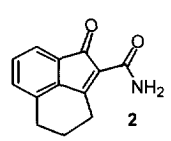
Abstract: Fibroblast growth factor receptor (FGFr) mediated signal transduction is implicated in vascular proliferative diseases and some cancers. We have identified methyl 1-oxo-3-phenyl-1*H*-indene-2-carboxylic ester as a small molecule inhibitor of the tyrosine kinase activity of FGFr-1, (IC_{50} = 5.1 μM). We report here the synthesis and structure-activity studies about this template core. Additionally, screening of this series against a panel of tyrosine kinases shows selective inhibition of FGFr. © 1997 Elsevier Science Ltd.

Fibroblast growth factor receptors (FGFr) are a family of transmembrane proteins involved in mitogenic signaling and regulation of a number of cellular processes.[1] Binding of fibroblast growth factor (FGF) to its cognate receptor (FGFr) initiates activation of the cytosolic tyrosine kinase (TK) domain for substrate binding and phosphorylation.[2] Evidence suggests that loss of regulatory control of FGFr is linked to a number of disease states including angiogenesis, restenosis following coronary angioplasty, rheumatoid arthritis, and some types of cancers.[3] Furthermore, FGFr may stimulate production of plasminogen activator, mediating migration of tumor cells into normal tissues.[3] Such indications have prompted interest in controlling signaling pathways mediated by this class of proteins. This objective is complicated by the considerable structural homology within the catalytic domain of FGFr with other receptor TKs.[4] Despite this, there have been several recent reports of selective inhibitors of this enzyme.[5]

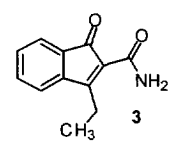
Upon screening our compound library for small molecule inhibitors of FGFr, we identified a number of 1-oxo-3-substituted-1*H*-indene-2-carboxamides (**1-3**) as moderately potent inhibitors of the TK activity (hereon FGFr, Table 1). These compounds also exhibited selective inhibition of FGFr relative to platelet derived growth factor receptor tyrosine kinase (PDGFr). Herein, we report the synthesis, characterization, and structure-activity relationships (SAR) of a series of 1-oxo-3-aryl-1*H*-indene-2-carboxylic acid derivatives.



1



2



3

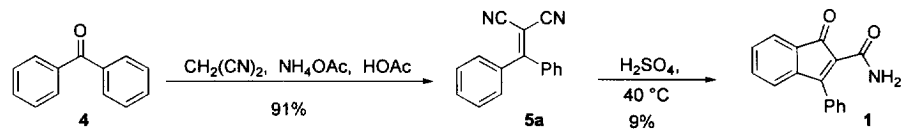
Table 1. Screening Leads (IC_{50} or % inhib at 50 μM; n=2).

NO.	FGFr[6]	PDGFr[6]	c-Src[7]
1	4.7 μM	25 %	3.6 μM
2	4.2 μM	25 %	48 %
3	9.2 μM	8.7 %	53 %

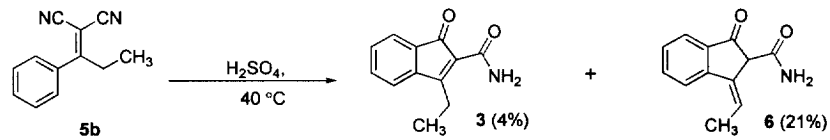
CHEMISTRY

The initial method[8] used to prepare **1** is shown in Scheme 1. Benzophenone (**4**) was readily converted to the corresponding malononitrile (**5a**) and cyclized in sulfuric acid to the desired compound in 9% yield. Using this method to prepare **3** gave a mixture of the desired compound and the β,γ-unsaturated compound, **6**, consistent with previous reports (Scheme 2).[8] The two indenones were separated by repeated crystallization to afford **3** (4%) and **6** (21%).

SCHEME 1



SCHEME 2

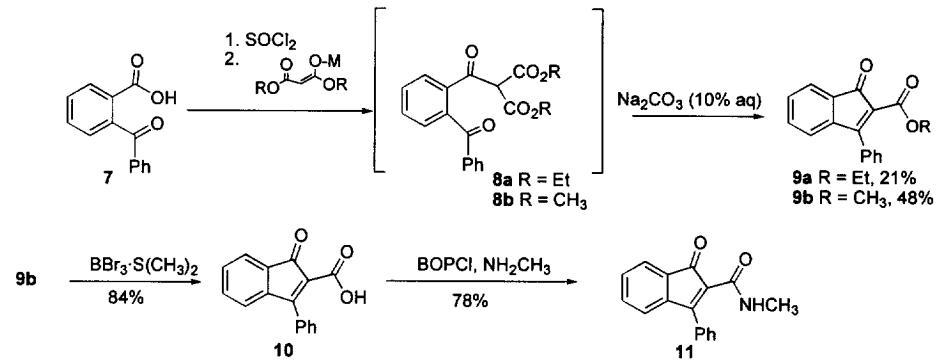


Although this method provides a direct route to 3-substituted analogs of **1**, it offers little flexibility toward investigating the SAR of the carboxylic moiety. Moreover, acid catalyzed cyclization of bis-aryl malononitriles generates a mixture of regioisomeric compounds,[9] complicating SAR studies of the 3-substituent. For these reasons, we sought a more general method to enable a broader SAR study of **1**.

We believed that ethyl 1-oxo-3-phenyl-1*H*-indene-2-carboxylic ester (**9a**) previously reported by Burger,[10] would be an ideal intermediate in route to substituted congeners in this series (Scheme 3). Thus, 2-benzoylbenzoic acid (**7**) was converted to the diethyl malonate adduct (**8a**) using thionyl chloride followed by treatment with potassium diethyl malonate. Treatment of unpurified **8a** with aqueous Na_2CO_3 afforded **9a** in 21% yield. In our hands, higher and more reproducible yields (48%) of the methyl ester, **9b**, were obtained by substituting sodium dimethyl malonate. While the carboxylic acid, **10**, has been prepared previously via an acid catalyzed cyclization of **8**,[10] we found this reaction unreliable and consequently investigated other routes. Aqueous hydrolysis of **9b** to **10** under acidic or alkaline conditions was unsuccessful, returning only **7**.[11]

Demethylation of **9b** was finally achieved using BBr_3, albeit in low yield (10%). A much improved conversion of **9b** to **10** (84%) was achieved using BBr_3•SMe_2.[12] Conversion of **10** to the substituted amide (**11**) was accomplished with standard BOPCl coupling conditions.[13]

SCHEME 3



Substituted 2-benzoyl benzoic acids were prepared from the appropriately substituted phthalic anhydride, according to published procedures.[14] In several cases, Grignard addition to substituted phthalic anhydrides afforded a mixture of regioisomers. Following conversion to the indenones, the ratio of isomers was determined by HPLC and ^{1}H NMR. Structural assignments of compounds **20** and **21** were confirmed by single crystal X-ray diffraction, and NMR experiments, respectively.

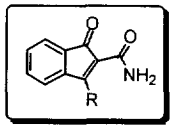
RESULTS AND DISCUSSION

Competition Experiments: Our initial experiments were designed to better understand the mechanistic aspects of the inhibitory activity observed within this class of compounds. The known utility of α,β–unsaturated systems as enzyme alkylating agents coupled with the propensity of similar compounds to undergo 1,4-addition reactions[11] led us to suspect that **1** inhibited FGFr via a general alkylation mechanism.[15] By carrying out the enzyme assay[6] in the presence of various concentrations of ATP, we showed that **1** is a reversible, ATP-competitive inhibitor of FGFr. Additionally, the inhibitory activity of **1** was unaffected by addition of exogenous DTT (chosen to mimic nucleophilc residues) to the assay cocktail. Taken together, these results suggest that a general alkylation mechanism is unlikely.

Structure-Activity Relationships: We evaluated the SAR of **1** by dissecting the molecule into three zones. We first examined substitution at the 3-position (Tables 1 and 2), followed by functional variation of the carboxyl group (Table 3), and finally substitution within the indenone core template (Tables 4 and 5). In order to ascertain enzyme selectivity, inhibitors were screened against PDGFr and c-Src.

In general, 3-alkyl substituted congeners were equipotent FGFr inhibitors compared to 3-aryl substituted derivatives (Tables 1 and 2). The alkyl substituted compounds, however, showed a slightly better enzyme selectivity profile (Table 1). Replacement of the 3-phenyl in **1** with a *para*-substituted aryl resulted in a slight decrease in FGFr inhibition and provided little variation in the selectivity profile (**12**, **13** Table 2).

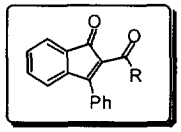
Table 2. SAR of 3-aryl derivative of **1**.



NO.	R	FGFr	PDGFr	c-Src
		IC_{50} (μM) or % inhib at 50 μM		
1	Ph	4.7	25%	3.6
12	4-Cl Ph	5.4	27%	9.6
13	4-OMe Ph	10.6	23%	4.0

Conversion of the 2-carboxamido substituent in **1** to the methyl ester (**9b**) maintains FGFr activity while increasing selectivity for FGFr approximately eight-fold (Table 3). Demethylation of **9b** to carboxylic acid **14** reduces FGFr activity five-fold and eliminates c-Src activity. Similar values were obtained for the *N*-methyl amide **11**. The more bulky *N,N*-dimethyl amide **15** is inactive in all enzyme assays, as is the solubilized *N*-(*N',N'*-diethylaminoethyl) analog **16**. Similar trends were observed with carboxamide derivatives of **12**.

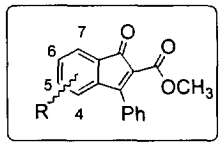
Table 3. SAR of carboxylic acid derivatives.



NO.	R	FGFr	PDGFr	c-Src
		IC_{50} (μM) or % inhib at 50 μM		
1	NH_2	4.7	23%	3.6
9b	OCH_3	5.1	19%	25
14	OH	25	9%	7%
11	$NHCH_3$	21	24%	16%
15	$N(CH_3)_2$	10%	10%	1%
16	$N(CH_2)_2NEt_2$	21%	8%	11%

Substitution at the 4, 5, or 6 positions of the benzenoid ring are well tolerated with small variations in selectivity (**17-20**, Table 4). The 7-methyl congener **21**, shows a reduction in FGFr activity. The heterocyclic derivative (**22**) affords similar FGF inhibition while enzyme selectivity is reduced.

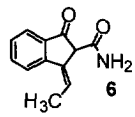
Table 4. SAR of the benzenoid ring.



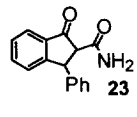
NO.	R	FGFr	PDGFr	c-Src
		IC_{50} (μM) or % inhib at 50 μM		
1	H	5.1	19%	25
17	5-CH_3/6-CH_3 (1:1)	6.4	20%	33
18	5-Cl/6-Cl(1:1)	6.2	25%	10%
19	5-NO_2/6-NO_2 (1:1)	10	19%	8.3
20	4-OCH_3	15	19%	13
21	7-CH_3	44	11%	38%
22	6-aza	6.8	27%	7.6

Alteration of the α, β-unsaturated ketone moiety either via isomerization (**6**), hydrogenation (**23**) or 1, 2-reduction (**24**) abolished all kinase activity (Table 5). These results suggest that a planar core template is critical for enzyme potency.

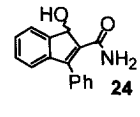
Table 5. SAR of the α, β-unsaturated indenone (IC_{50} or % inhib at 50 μM; n = 2).



6



23



24

NO.	FGFr	PDGFr	c-Src
6	26%	8%	3%
23	23%	16%	8%
24	29%	10%	27%

CONCLUSIONS

We have prepared a series of 1-oxo-3-aryl-1*H*-indene-2-carboxylic acid derivatives by modifying previously reported chemistry. Kinetic studies indicate that compounds of this series are reversible, ATP competitive inhibitors of the tyrosine kinase domain within FGFr . SAR studies within this series indicate that **9b** represents the optimal substitution pattern for potency and selectivity for FGFr relative to that for c-Src and PDGFr.

ACKNOWLEDGMENTS

We thank Brian Tobias and Lee Schultz for carrying out the NOE and INEPT studies, and John Rubin for solving the crystal structure of compound **20**.

REFERENCES

1. (a) Jaye, M.; Schlessinger, J.; Dionne, C. A. *Biochemica et Biophysica Acta* **1992**, *1135*, 185. (b) Fantl, W. J.; Johnson, D. E.; Williams, L. T. *Ann. Rev. Biochem.*, **1993**, *62*, 453.

2. Mohammadi, M.; Schlessinger, J.; Hubbard, S. R. *Cell* **1996**, *86*, 577.

3. (a) Klagsburn, M.; Edelman, E. R. *Arteriosclerosis* **1989**, *9*, 269. (b) Brem, H. Klagsburn, M. in *Oncogenes and Tumors Suppressor Genes in Human Malignancies;* Benz, C. C.; Liu, E. T. Ed. Kluwer Academic: Boston, 1993.

4. Cadena, D. L.; Gill, G. N. *FASEB J.* **1992**, *6*, 2332.

5. Wellstein, A. Czybayko, F. *Breast Cancer Res. Tr.* **1996**, *38*, 109. Fry, D. W. Nelson, J. M. *Anti-CancerDrug Design* **1995**, *10*,607.

6. (a) Lu, G. H.; Conners, R.; Panek, R. Presented at FASEB, Spring 1994, Anaheim, CA, Abstract 253. (b) Lu, G. H.; Connors, R.; Panek, R. *FASEB J.* **1994**, *37*, A49.

7. c-Src tyrosine kinase enzyme inhibition assays are identical to the previously reported assay for v-Src (Thompson, A. M.; Fry, D. W.; Kraker, A. K.; Denny, W. A. *J. Med Chem.* **1994**, *37*, 598), with the exception of the enzyme source and the ATP concentration (40 μM).

8. Campaigne, E.; Bulbenko, W. E.; Kreighbaum, W. E.; Maulding, D. R. *J. Org. Chem.* **1962**, *27*, 4428.

9. Moussa, H. H.; Zaki, D. *Pol. J. Chem.* **1981**, *55*, 2445.

10. Yost W. L., Burger, A. *J. Org. Chem.* **1950**, *15*, 1113.

11. (a) Koelsch, C. F. *J. Org. Chem.* **1960**, *25*, 2088. (b) Jourdan, G. P.; Dreikorn, B. A.; Hackler, R. E.; Hall, H. R. Arnold, W. R. ACS Symp. Ser. (1991), 443 (Synth. Chem. Agrochem. 2) 556-574.

12. A representative procedure: To a suspension of $BBr_3{\cdot}S(CH_3)_2$ (468 mg, 1.5 mmol) in anhydrous 1,2-dichloroethane (5 mL) was added a solution of **9b** (265 mg, 1 mmol) in 1,2-dichloroethane (3 mL). The resulting mixture was heated to reflux for 2 h, cooled and quenched by slow addition of saturated $NaHCO_3$ (aq) with cooling. Crude **10** was isolated following acidification of the aqueous layer to pH 2 (6 N HCl), extraction with CH_2Cl_2 and concentration of the organic layer. An analytically pure sample of **10** was obtained following recrystallization from methyl *t*- butyl ether.

13. For **11**: ^{1}H NMR ($CDCL_3$): δ 7.87 (bd s, 1H), 7.54-7.51 (m, 4H), 7.50-7.45 (m, 3H), 7.38-7.35 (m, 2H), 7.09-7.06 (m, 1H), 2.82 (d, J = 4.9 Hz, 3H). IR (KBr) cm^{-1} 3429 (bd), 2973, 2925, 1625, 1557, 1542.

14. Mertens, A.; Zilch, H.; Konig, B.;Schafer, W.;Poll, T.; Kampe, W.; Seidel, H.; Lesser, U.; Leinert, H. *J. Med. Chem.* **1993**, *36*, 2526 and references cited therein.

15. For examples and discussion see: *Structure-Based and Mechanism-Based Enzyme Inhibitor Drug Design and Drug Action.* Silverman, Richard B. Ed. Pergamon: Oxford, UK 1996.

(*Received in USA* 16 *September* 1997; *accepted* 14 *October* 1997)