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Bioorganic & Medicinal Chemistry Letters
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Bioorganic & Medicinal Chemistry Letters 14 (2004) 187–190

Design and synthesis of aminopropyl tetrahydroindole-based indolin-2-ones as selective and potent inhibitors of Src and Yes tyrosine kinase

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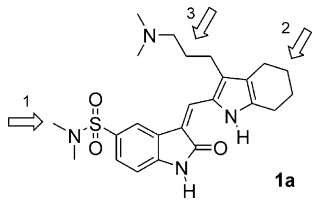
Received 14 May 2003; revised 20 September 2003; accepted 27 September 2003

Abstract—A novel series of substituted 3-[3-(aminopropyl)-4,5,6,7-tetrahydro-1*H*-indol-2-ylmethylene]-1,3-dihydro-indole-2-ones was discovered as potent inhibitors of the non-receptor tyrosine kinase Src and Yes. A structure–activity relationship was developed in order to optimize their potency and selectivity. Syntheses of these compounds are also described herein.

Src and its close kinase sub-family members, such as Yes, Fyn, Lyn and Lck, are non-receptor protein tyrosine kinases which function as early upstream signal transduction proteins.[1,2] Src and Yes are widely expressed and activated in a great number of tumor types. In particular, Src has been identified as a prototype oncogene and is considered to be a validated therapeutic target for cancer, osteoporosis and other diseases.[3] Extensive research has been directed to discover Src inhibitors and a number of them have been reported,[4–11] though none has been reported in clinical trials to date.[12]

Screening of a proprietary compound collection showed several tetrahydroindole based indolinones as potent and selective Src inhibitors. In particular, 3-[3-(3-dimethylamino-propyl)-4,5,6,7-tetrahydro-1*H*-indol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid methylamide (**1a**) was identified as a potent inhibitor of Src kinase activity (0.1 μM in an enzymatic assay and a functional cellular phosphorylation assay) with over 10-fold selectivity against closely related kinases such as Lck and PDGFRβ. The present study reports the synthesis and initial structure–activity relationship (SAR) on this novel class of compounds. Our chemistry efforts were focused on (1) the C-5 position of the oxindole ring, (2) the tetrahydroindole moiety and (3) the 3′-amino side chain as shown in Figure 1.

In general, the syntheses consist of three steps: (1) preparation of substituted oxindole core (2) preparation of functionalized pyrrole aldehydes (3) aldol-condensation of the two moieties under basic conditions[13] to furnish final indolinones **1**, **2**, **3**, and **4**. Most of the oxindoles with C-5 substitution were either commercially available or prepared by published methods.[14] Oxindole sulfones **7** and sulfonamides **8** were prepared by sulfonation of 2-oxindoles **5** into **6** followed by alkylation or amidation of the sulfonyl chloride group of **6**, respectively (Scheme 1). Compound **14** was synthesized via procedures illustrated in Scheme 1. Intermediate **11** was obtained by reduction of **9** to its sodium methylsulfonite **10** followed by reaction with 4-bromomethyl nitrobenzene. Reduction of the nitro group of **11** led to **12**, which was condensed



1a

Figure 1. Lead compound and SAR focus.

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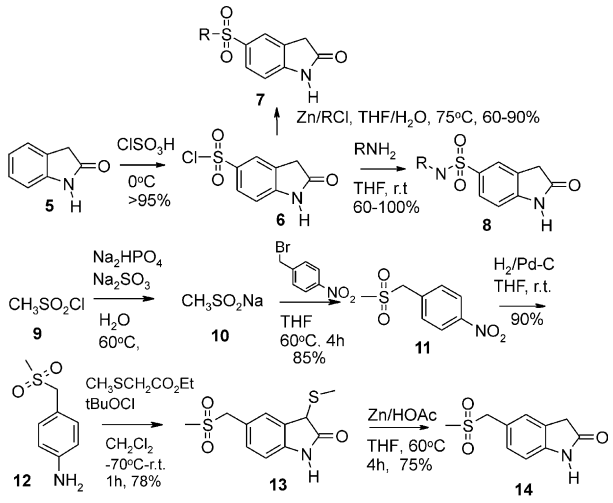
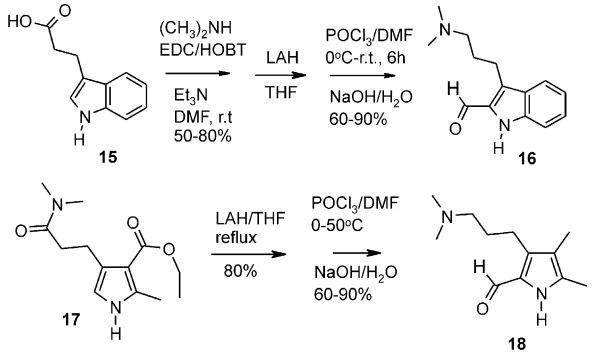
doi:10.1016/j.bmcl.2003.09.069



Scheme 1. Synthesis of substituted indolin-2-ones.



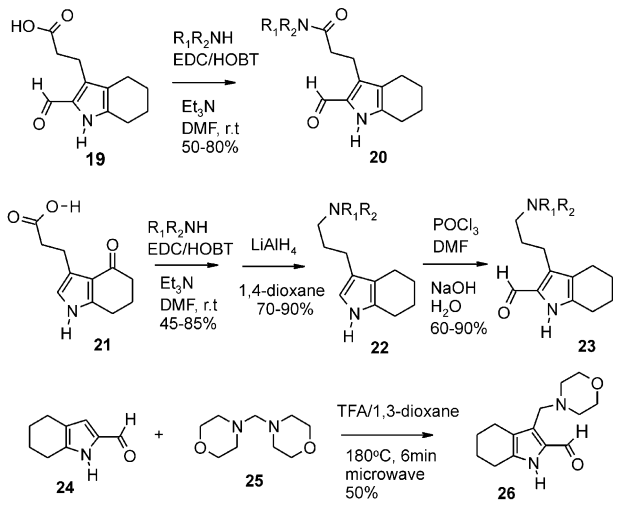
Scheme 2. Synthesis of analogues of tetrahydroindole aldehydes.

with ethyl(methylthio)acetate to produce the key intermediate **13**. Reductive cleavage of the methylthio group of **13** furnished **14**.

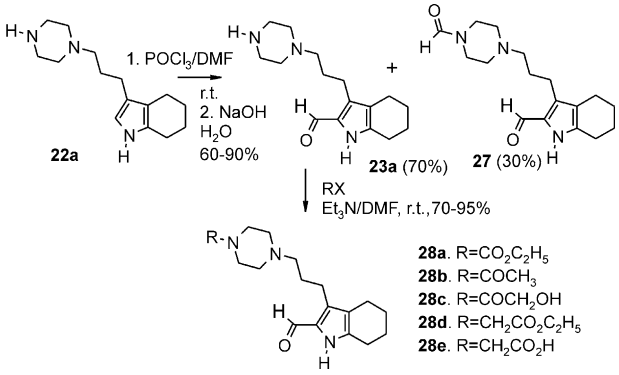
Analogues of 4,5,6,7-tetrahydroindole-2-carbaldehydes **16** were made by amidation, reduction and Vilsmeier formylation of commercially available **15**. **18** was synthesized by reducing both the amido and ethoxy carbonyl groups of **17**[14] followed by formylation (Scheme 2).

Syntheses of derivatives of tetrahydroindole aldehydes are illustrated in Scheme 3, where R_1R_2N represents different amines. 3-(2-Formyl-4,5,6,7-tetrahydroindol-3-yl)-propionamides **20** was derived from **19**.[13] Compounds **22** were made from intermediate **21**[15,16] via amidation and reduction. Formylation of **22** afforded **23**. Mannich type of reaction to attach a morpholinomethyl moiety to the C-3′ position of 4,5,6,7-tetrahydroindole-2-carbaldehydes **26** was achieved in a microwave reactor. The terminal amino group of piperazine moiety in **23a** was further diversified by amidation or alkylation to furnish **28a–e** (Scheme 4).

Finally, different oxindoles prepared according to Scheme 1 were reacted with the tetrahydroindoles **23** ($R_1 = R_2 =$ methyl) in Scheme 3 to yield series **1**. Series **2** compounds were derived from the reaction of the 5-*N*-methylsulfonamide oxindole with substituted pyrrole or



Scheme 3. Synthesis of functionalized tetrahydroindole aldehydes.



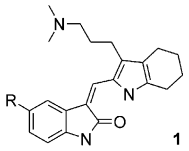
Scheme 4. Diversification of the piperazine moiety.

indole aldehyde shown in Scheme 2. Series **3** and **4** were prepared from the two oxindoles condensed with the tetrahydroindole aldehydes in Schemes 3 and 4.

An initial SAR study revealed that the substituents at the C-5 position of the oxindole core had a dramatic impact on the inhibitory activity against Src kinase (Table 1). Larger alkyl secondary sulfonamides (**1c–f**) and tertiary sulfonamides (**1g**) reduced Src inhibitory activity. Replacing the sulfonamide with a sulfonyl group of similar size (**1i**) slightly improved Src potency. Varying the size of the alkyl led to weaker Src inhibitors (**1h**,**j**). These results depict that methylsulfonamide of **1a** or ethylsulfonyl of **1i** have favorable size and that the acidic proton of sulfonamide is not critical to Src activity. However, the sulfone moiety was essential to potency towards Src. Its removal (**1l**) or replacement with carbonyl (**1m**,**n**), phenyl (**1o**), fluorine (**1p**), or methoxy (**1q**) all diminished Src inhibitory activity. Insertion of a methylene between the sulfonyl and oxindole abolished Src activity (**1k**), suggesting that the sulfonyl group needs to be directly attached to the oxindole core.

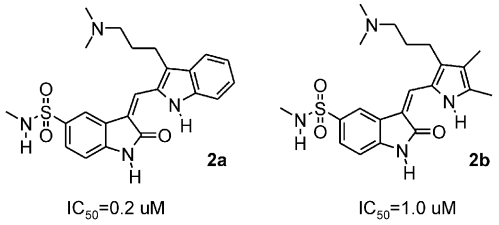
The tetrahydroindole moiety was found to be favorable for Src activity. Replacing it with indole (**2a**) or opening the six-membered ring (**2b**) decreased the inhibitory potency towards Src kinase activity (Fig. 2).

Table 1. Biochemical Src kinase inhibitory activity of compounds **1a–q**



Compd	R	Src IC_{50} (μM)*
1a	SO_2NHCH_3	0.10
1b	SO_2NH_2	0.6
1c	$SO_2NHCH_2CH_2OH$	0.24
1d	$SO_2NHCH(CH_3)_2$	0.23
1e	SO_2NHCH_2(4-F–Ph)	1.2
1f	SO_2NH(2-Cl–Ph)	0.3
1g	$SO_2N(CH_3)_2$	2.6
1h	SO_2CH_3	0.3
1i	$SO_2C_2H_5$	0.06
1j	$SO_2CH(CH_3)_2$	0.23
1k	$CH_2SO_2CH_3$	10
1l	H	3.3
1m	CO_2H	0.7
1n	CO_2NHCH_3	1.1
1o	Ph	2.3
1p	F	0.5
1q	OCH_3	1.0

*IC_{50} values were determined by at least two separate tests with deviation less than 20% and are reported as mean values.



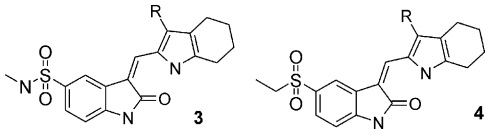
IC_{50}=0.2 uM IC_{50}=1.0 uM

Figure 2. Structure of **2a** and **2b** and their Src kinase inhibitory activity.

With the optimized oxindole substituents (5-ethylsulfone and 5-methylsulfonamide) and the tetrahydroindole moiety fixed, we further examined variations on the C-3′ position of the tetrahydroindole core (Table 2). The results indicated that the C-3′ side chain was required since its deletion impaired the activity by over 10-fold (**3a** and **4a**, compared to **1a** and **1i**, respectively). Replacing the basic head group by an acidic group reduced potency (**4b**). However, there is no obvious trend regarding the length or basicity of the C-3′ substituents. For example, shortening the linker improved activity by 10-fold in **3c** over the initial lead **1a**, but did not affect **4c** compared to **1i**. Replacing the amino chain by amido chain afforded more potent compounds **3d** and **4d** as well as less potent compounds **3e**, **4e**. Varying the basicity of the side chain has little effect on Src activity as well (**3f–h**, vs **1a**). Further diversification of piperazylpropyl chain produced mixed results. Some (**3l–o**) are more potent than **1a**, while others (**3i–k**) are weaker, with the largest difference as high as 20-fold in inhibiting Src enzymatic activity (Table 2).

Most compounds were also evaluated for their inhibitory activity against tyrosine phosphorylation by Yes,

Table 2. Biochemical Src kinase inhibitory activity of compounds **3a–p** and **4a–p**



Compd	R	Src IC_{50} (μM)*
3a	H	2.1
4a	H	0.7
4b	$(CH_2)_2CO_2H$	0.4
3c	$CH_2N(CH_2CH_2)_2O$	0.01
4c	$CH_2N(CH_2CH_2)_2O$	0.07
3d	$(CH_2)_2CONH_2$	0.03
4d	$(CH_2)_2CONH_2$	0.04
3e	$(CH_2)_2CON(CH_2CH_2)_2NCH_3$	0.3
4e	$(CH_2)_2CON(CH_2CH_2)_2NCH_3$	0.5
3f	$(CH_2)_3N(CH_2CH_2)_2O$	0.06
4f	$(CH_2)_3N(CH_2CH_2)_2O$	0.15
3g	$(CH_2)_3N(CH_2CH_2)_2NCH_3$	0.07
4g	$(CH_2)_3N(CH_2CH_2)_2NCH_3$	0.21
3h	$(CH_2)_3N(CH_2CH_2)_2NH$	0.2
3i	$(CH_2)_3N(CH_2CH_2)_2NCO_2C_2H_5$	0.3
4i	$(CH_2)_3N(CH_2CH_2)_2NCO_2C_2H_5$	0.8
3j	$(CH_2)_3N(CH_2CH_2)_2NCOCH_3$	0.3
4j	$(CH_2)_3N(CH_2CH_2)_2NCOCH_3$	0.4
3k	$(CH_2)_3N(CH_2CH_2)_2NCOCH_2OH$	0.4
4k	$(CH_2)_3N(CH_2CH_2)_2NCOCH_2OH$	0.22
3l	$(CH_2)_3N(CH_2CH_2)_2NCH_2CO_2C_2H_5$	0.07
3m	$(CH_2)_3N(CH_2CH_2)_2NCH_2CO_2H$	0.02
4m	$(CH_2)_3N(CH_2CH_2)_2NCH_2CO_2H$	0.07
3n	$(CH_2)_3N(CH_2CH_2)_2CHOH$	0.05
4n	$(CH_2)_3N(CH_2CH_2)_2CHOH$	0.07
3o	$(CH_2)_3N(CH_2CH_2)_2NCH_2CH_2OH$	0.03
4o	$(CH_2)_3N(CH_2CH_2)_2NCH_2CH_2OH$	0.07

*IC_{50} values were determined by at least two separate tests with deviation less than 20% and are reported as mean values.

Lck, Fyn, VEGFR2, FGFR1, or PDGFRβ according to previously reported methods.[13,17] Inhibition of cellular Src function was evaluated using the actin ring assay which assesses the Src-dependent cytoskeletal reorganization of podosome rosettes in fibroblast cells transformed with an activated mutant of Src.[17,18] Selected data for the most potent compounds are listed in Table 3. It shows that this class of compounds is very potent against Yes as well as Src. But they displayed about 10-fold selectivity for Src over Lck and sometimes Fyn. The selectivity with respect to VEGFR2 and FGFR1 was moderate in some cases, but the potent compounds with amido side chains (**3d**, **4d**) were not very selective against FGFR1. Most compounds exhibited high selectivity for Src over PDGFRβ.

Although most compounds in Table 3 are more potent in Src biochemical assay than the initial lead **1a**, this did not necessary translate into improved cellular potency. In fact, most of these compounds were much less potent in cells than **1a** and only the close analogues **3f–g** have comparable cellular potency. The lack of cellular potency for enzymatically potent Src inhibitors was also observed in other series of compounds.[5,6,10,11] It is interesting to note that the zwitterion **3m** was inactive in cells (perhaps because of poor cell membrane permeability), while its ester analogue (**3l**) had moderate cellular activity. All of the inhibitors with relatively good

Table 3. Biochemical selectivity and cellular Src activity of selected compounds

Compd	Biochemical activity (IC_{50}, μM)[a]							Cellular (IC_{50}, μM)[a]
	Src	Yes	Lck	Fyn	VEGFR2	FGFR1	PDGFRβ	
1a	0.1	0.01	1.6	0.06	1.1	0.7	3.3	0.1
1i	0.06	0.02	0.2	0.3	2.7	0.4	2.5	0.5
3c	0.01	0.04	0.08	0.4	0.06	0.2	NT[b]	1.0
4c	0.07	0.16	0.8	1.4	0.1	0.4	NT[b]	5.0
3d	0.03	0.01	0.1	0.05	0.5	0.07	6.6	>10
4d	0.04	0.03	—	0.2	0.3	0.08	5.1	>10
3f	0.06	0.03	0.7	0.7	2.3	1.7	18	0.1
3g	0.07	0.01	0.7	0.8	0.3	1.2	10	0.1
4g	0.16	0.03	0.6	1.2	2.6	NT[b]	11	0.2
3l	0.07	0.02	0.6	0.5	0.5	0.08	>20	0.9
3m	0.02	0.01	0.1	0.4	0.4	0.2	8	>10
4m	0.07	0.01	0.4	0.4	0.8	0.4	13	10
3n	0.05	0.01	0.3	0.2	7.1	3.4	4.7	0.9
4n	0.07	0.02	0.7	0.6	0.5	0.5	2.3	0.8
3o	0.03	0.01	0.4	0.1	0.4	0.6	5.6	3
4o	0.07	0.04	0.5	0.4	0.4	1.1	9	1.7

[a] IC_{50} values were determined by at least two separate tests with deviation less than 20% and are reported as mean values.
[b] NT, not tested.

cellular activity (**1a**, **1i**, **3f**, **3g**, **4g**, $IC_{50} < 0.5$ μM) contained a basic (3-amino)propyl side chain attached to the C-3′ of the tetrahydroindole moiety, suggesting that it confers to cellular potency.

In conclusion, we have explored substituted 3-[3-(aminopropyl)-4,5,6,7-tetrahydro-1*H*-indol-2-ylmethylene]-1,3-dihydro-indole-2-ones as potent and selective inhibitors of Src and Yes kinases. Initial SAR study suggested that the 5-sulfonyl substitution, tetrahydroindole ring, and 3-aminopropyl side chain of the initial lead **1a** are all critical to biochemical and cellular potency and kinase selectivity for Src.

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