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1,5-Disubstituted indole derivatives as selective human neuronal nitric oxide synthase inhibitors

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ABSTRACT

A series of 1,5-disubstituted indole derivatives was designed, synthesized and evaluated as inhibitors of human nitric oxide synthase. A variety of flexible and restricted basic amine side chain substitutions was explored at the 1-position of the indole ring, while keeping the amidine group fixed at the 5-position. Compounds having *N*-(1-(2-(1-methylpyrrolidin-2-yl)ethyl)- (**12**, (***R***)-**12**, (***S***)-**12** and **13**) and *N*-(1-(1-methylazepan-4-yl)- side chains (**14**, **15**, (−)-**15** and (**+**)-**15**) showed increased inhibitory activity for the human nNOS isoform and selectivity over eNOS and iNOS isoforms. The most potent compound of the series for human nNOS (IC_{50} = 0.02 μM) (***S***)-**12** showed very good selectivity over the eNOS (eNOS/nNOS = 96-fold) and iNOS (iNOS/nNOS = 850-fold) isoforms.

Nitric oxide (NO), which is produced from L-arginine by the nitric oxide synthase (NOS) enzyme, is one of the most studied neuromodulators.[1,2] NOS consists of three isoforms: neuronal NOS (nNOS), is a constitutive form expressed in neuronal cells and thought to play a role in neurotransmission and the endothelial isoform (eNOS), is also a constitutive form plays a role in regulating vascular tone and platelet aggregation. The third isoform is an inducible form (iNOS) found in macrophage cells, which produce NO during bacterial infection or tumor cell cytolysis. Overproduction of NO by the individual NOS isoforms, such as nNOS and/or iNOS plays an important role in the treatment of several disorders including septic shock, stroke, neurodegenerative disorders (Parkinson's, ALS, MS) and pain (migraine, CTTH, visceral and neuropathic).[3] The selective inhibition of nNOS and/or iNOS over the eNOS isoform is required in order to avoid the cardiovascular liabilities associated with eNOS inhibition.[4]

Developing small molecule selective NOS inhibitors is an ongoing challenge, due to the high similarity of the active sites of the three isoforms, where 16 out of 18 residues within 6 Å of the substrate-binding pocket are identical.[5] Structure-based inhibitor design became a useful tool with the availability of crystal structures of NOS enzymes with the only exception of human nNOS isoform.[6] Early NOS inhibitor design that focused mainly on L-arginine (substrate) type compounds targeting the substrate binding site produced only nonselective NOS inhibitors. However, later generations of peptidic and nonpeptidic NOS inhibitors targeting the arginine binding site or Biopterin co-factor site as well as dimerization inhibitors have been shown to be more potent and selective among the NOS isoforms.[7–9]

The pharmacophore model **1** (Fig. 1) that describes the arginine binding site of the NOS enzyme contains an amidine group (guanidine isostere) and a basic amine group, attached to the indole core (central aryl scaffold). The amidine group makes an important bidentate interaction with the conserved glutamic acid residue; whereas the basic amine is known to improve potency and selectivity among the NOS isoforms for compounds with a thiophene amidine group (**2** and **3**).[10–12] An increased potency and selectivity was observed for nNOS isoform over eNOS and iNOS isoforms with an amino substitution on thiophene amidine compounds containing either a benzothiazole or dihydroquinoline core.[10,11] Our design strategy is based on attaching a basic amine side chain and thiophene amidine group onto an indole core, which is an important structural component in many pharmaceutical agents and also referred to as 'privileged structure' (capable of binding to many receptors).[13] In our continued search for new small molecule drug-like selective nNOS inhibitors,[10] herein we report the design and synthesis of a series of 1,5-disubstituted indole derivatives and their biological activity evaluations against all human NOS isoforms.

Synthesis of 1,5-disubstituted indole derivatives was carried out according to the general procedures described in Schemes 1–6. The 3-carbon side chain was introduced at the 1-position of 5-nitro-1*H*-indole using a two-stage alkylation procedure (Scheme 1). The

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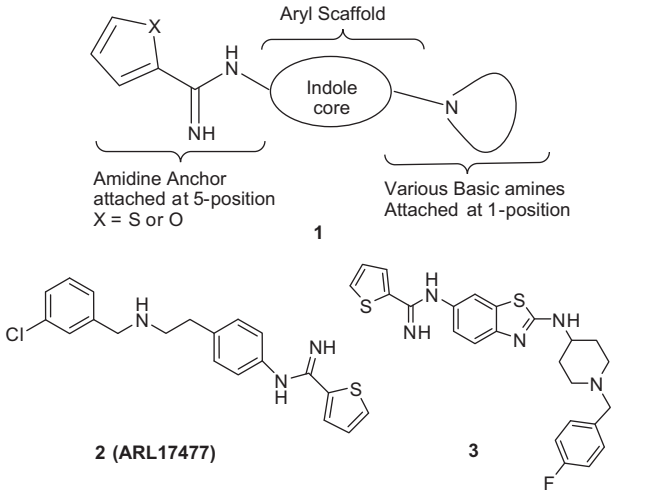
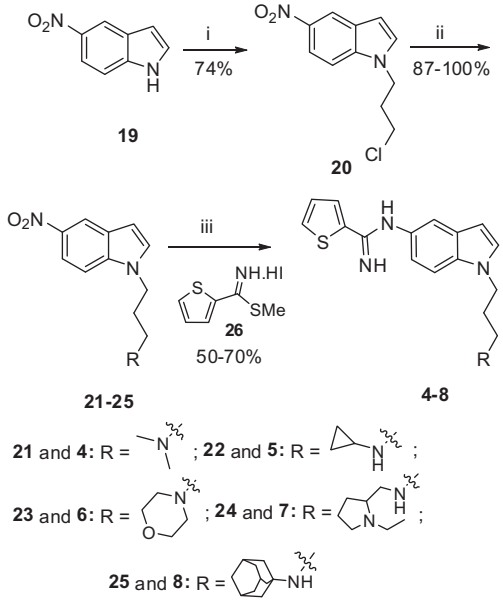


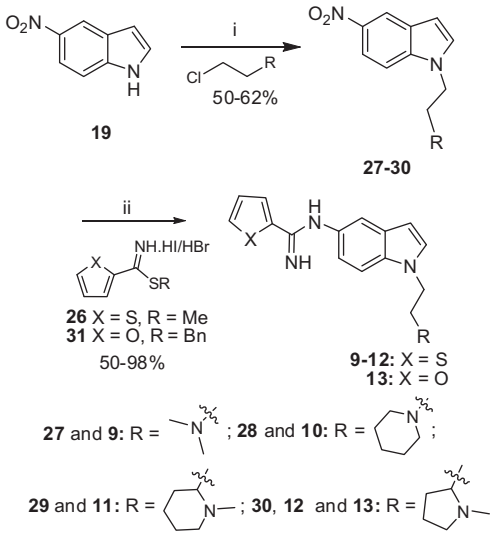
Figure 1. Pharmacophore model (**1**) for selective nNOS inhibitor design. Literature thiophene amidine compounds with basic amine side chain (**2** and **3**).

alkylation of 5-nitro-1*H*-indole (**19**) with 1-chloro-3-iodopropane under basic conditions gave the chloro intermediate **20**. The chloro group in compound **20** was substituted with various acyclic or cyclic amines to obtain the intermediates **21–25** in excellent yield. The nitro group was then reduced to the corresponding amino group under hydrogenation conditions and coupled with the thiophene-2-carbimidothioate **26** to provide the final compounds **4–8**.[14]

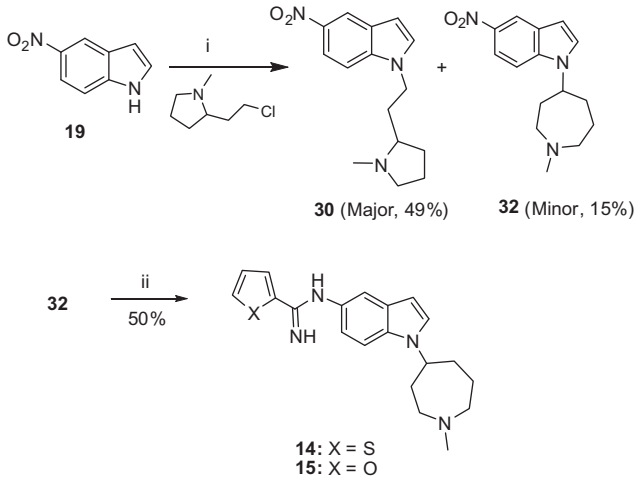
The alkylation of 5-nitro-1*H*-indole (**19**) with various 2-chloroethyl amino derivatives in presence of potassium carbonate gave intermediates **27–30** (Scheme 2). The nitro group was reduced to amino group and coupled with either thiophene-2-carbimidothioate **26** or furan-2-carbimidothioate **31** to give the final compounds **9–13**.[15] During the alkylation reaction with 1-(2-chloroethyl)-2-methylpyrrolidine, rearrangement (ring opening/quarternization) was observed to obtain a 7-membered product **32** (15%) along with the desired product **30** (Scheme 3).[16] Product **30** and rearranged product **32** were separated by using silica gel column chromatography. The nitro group in compound **32** was reduced to the corresponding amino group as described above



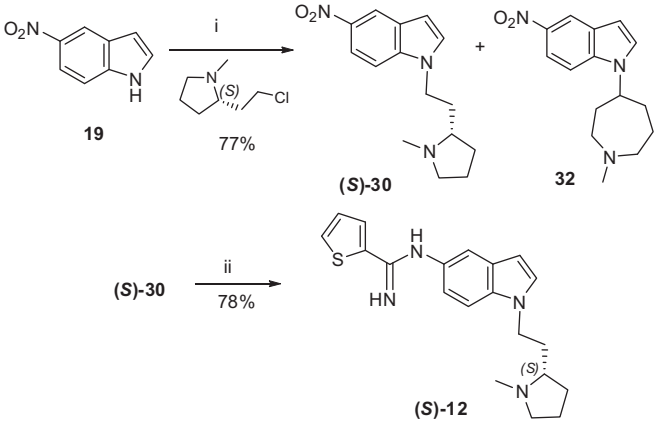
Scheme 1. (i) NaH, 1-chloro-3-iodopropane, DMF; (ii) RH, K_2CO_3, KI, AcCN, reflux; (iii) (a) Pd-C/H_2, EtOH, RT; (b) **26**, EtOH, RT.



Scheme 2. (i) K_2CO_3, DMF, 80 °C; (ii) (a) Pd-C/H_2, EtOH, RT; (b) **26** or **31**, EtOH, RT.

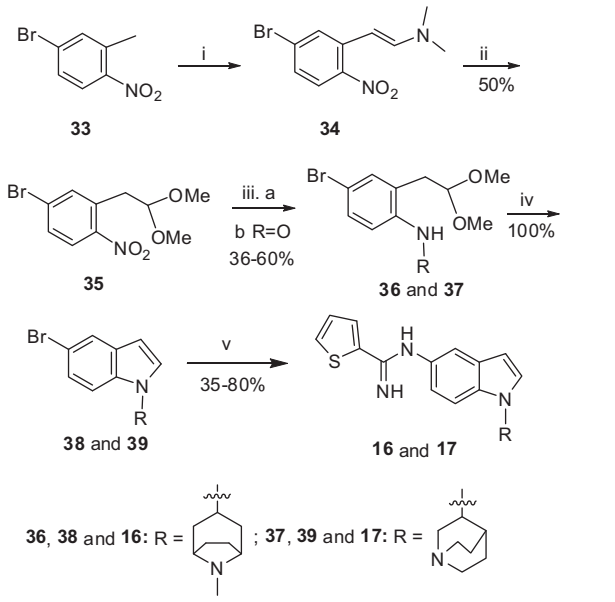


Scheme 3. (i) K_2CO_3, DMF, 80 °C; (ii) (a) Pd-C/H_2, EtOH, RT; (b) **26** or **31**, EtOH, RT.

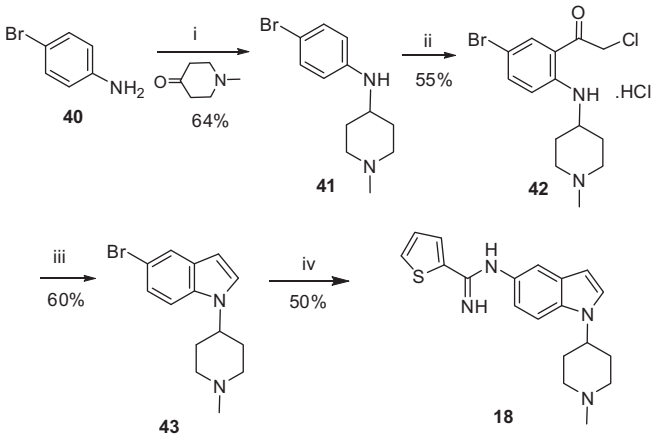


Scheme 4. (i) K_2CO_3, DMF, 80 °C; (ii) (a) Pd-C/H_2, EtOH, RT; (b) **26** or **31**, EtOH, RT.

and coupled to thiophene-2-carbimidothioate **26** and furan-2-carbimidothioate **31** to obtain the final compounds **14** and **15**, respectively.



Scheme 5. (i) DMFDMA, pyrrolidine, DMF, 110 °C; (ii) chlorotrimethylsilane, MeOH, reflux; (iii) (a) sodium dithionite, $NaHCO_3$, EtOH, reflux; (b) Na_2SO_4, $NaBH(OAc)_3$, AcOH, RT, (iv) 1 N HCl, MeOH, reflux; (v) (a) $Pd_2(dba)_3$, LiHMDS, P^tBu_3, THF, reflux; (b) **26**, EtOH, RT.



Scheme 6. (i) $NaBH(OAC)_3$, AcOH, DCE, 55 °C; (ii) chloroacetonitrile, BCl_3, DCE, reflux; (iii) $NaBH_4$, 1 M NaOH, EtOH, 0 °C; (iv) (a) $Pd_2(dba)_3$, LiHMDS, P^tBu_3, THF, reflux; (b) **26**, EtOH, RT.

Compound **12** was separated into its enantiomers **(*R*)-12** and **(*S*)-12** using chiral HPLC techniques.[17] The stereochemistry of the separated enantiomers was confirmed by an independent chiral synthesis using (*S*)-2-(2-chloroethyl)-1-methylpyrrolidine (Scheme 4).[18] Accordingly, 5-nitro-1*H*-indole (**19**) was treated with (*S*)-2-(2-chloroethyl)-1-methylpyrrolidine in presence of potassium carbonate to provide the chiral intermediate **(*S*)-30** along with the rearranged product **32**. The nitro group in compound **(*S*)-30** was converted into the corresponding amino group and coupled with the thiophene-2-carbimidothioate **26** to give the chiral **(*S*)-12**. Racemic compound **12** and its separated enantiomers **(*R*)-12** and **(*S*)-12** were co-injected with the synthesized chiral compound **(*S*)-12** to determine the stereochemistry of the separated enantiomers.[19] Compound **15** was also separated into its enantiomers **(−)-15** and **(+)-15** using chiral HPLC techniques,[20] but no further attempts were made to determine the stereochemistry of compounds **(−)-15** and **(+)-15** at this time.

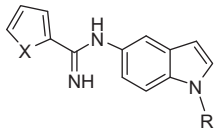
In contrast to the synthesis of 2- or 3-carbon flexible side chain analogs (**4**–**13**), the synthesis of rigid side chain analogs **16** and **17** was achieved by an indolization reaction using a modified Leimgruber–Batcho indole synthesis.[21] Condensation of substituted *o*-nitro toluene **33** with dimethylformamide dimethyl acetal in presence of pyrrolidine gave intermediate **34** (Scheme 5). The enamine **34** was converted into acetal **35** using chlorotrimethylsilane in methanol under refluxing conditions. The nitro group in compound **35** was converted into an amino group using sodium dithionite, followed by reductive amination with either 8-methyl-8-azabicyclo[3.2.1]octan-3-one or quinuclidin-3-one gave intermediates **36** and **37**.[22,23] The indolization reaction of intermediates **36** and **37** was carried under strong acidic conditions to provide the substituted 5-bromo-1*H*-indole derivatives **38** and **39** in quantitative yield. The bromo group in intermediates **38** and **39** was converted into the corresponding animo group using standard Buchwald-Hartwig amination reaction conditions,[24] followed by the coupling of the amine to the thiophene-2-carbimidothioate **26** provided the final compounds **16** and **17**, respectively.

A standard reductive amination reaction between 4-bromoaniline **40** and 1-methylpiperidin-4-one in presence of triacetoxyborohydride and acetic acid gave the intermediate **41** (Scheme 6). Intermediate **41** was chloroacetylated using chloroacetonitrile in presence of BCl_3 to obtain compound **42**.[25] The reductive cyclization (indolization) of compound **42** with sodium borohydride in presence of sodium hydroxide was carried out to provide the substituted 5-bromo-indole **43**. The indole derivative **43** was converted into final compound **18** using standard Buchwald–Hartwig amination, followed by coupling to the thiophene-2-carbimidothioate **26** as described above.

All compounds were converted into their corresponding dihydrochloride salts (except **7**, which is a trihydrochloride salt) to improve their water solubility,[26] and their inhibitory activities measured against all three human NOS isoforms (Table 1).[27] All compounds displayed sub-micromolar potencies for human nNOS isoform and good selectivity over the human eNOS and iNOS isoforms. Compound (***S***)-**12** was identified as the most potent nNOS inhibitor (IC_{50} = 0.02 μM) in the series with very good selectivity over eNOS (eNOS/nNOS = 96-fold) and iNOS (iNOS/nNOS = 850-fold) isoforms. Compounds with *N*-(1-(2-(1-methylpyrrolidin-2-yl)ethyl)- (**12**, (***R***)-**12**, (***S***)-**12** and **13**) and *N*-(1-(1-methylazepan-4-yl)- side chains (**14**, **15**, (−)-**15** and (**+**)-**15**) showed excellent selectivity for nNOS over eNOS and iNOS isoforms. Compound **9** with a 2-carbon linker was less selective (threefold for nNOS and eNOS, sixfold for iNOS) compared to the corresponding compound **4** with a 3-carbon linker. 2-Furanyl amidines **13** and **15** showed weaker activities (~2-fold for nNOS and eNOS isoforms), and (~2- to 6-fold for iNOS isoform) when compared to their corresponding 2-thiophene amidine compounds **12** and **14**, respectively. The separated enantiomers (***R***)-**12** and (***S***)-**12** did not show any significant difference in potency or selectivity among the NOS isoforms, when compared to the racemic mixture **12**. Similarly the enantiomers (−)-**15** and (**+**)-**15** also did not show any significant difference in potency or selectivity among the NOS isoforms in comparison to the racemate **15**.

In summary, we have designed and synthesized a series of 1,5-disubstituted indole derivatives, which showed sub-micromolar potencies for human nNOS with good selectivities over eNOS and iNOS isoforms. In general compounds with *N*-(1-(2-(1-methylpyrrolidin-2-yl)ethyl)- (**12**, **(*R*)-12**, **(*S*)-12** and **13**) or cyclic (**14**, **15**, **(−)-15**, **(+)-15**, **16**, **17** and **18**) side chains were shown to provide better selectivities for human nNOS over eNOS and iNOS isoforms. We have also shown that by varying the basic amine side chain, as much as 96- and 850-fold selectivity for human nNOS over eNOS and iNOS, respectively, can be achieved without reducing the po-

Table 1
Inhibition of human NOS enzymes by 1,5-disubstituted indole derivatives



Compound	X	R	nNOS IC_{50} (μM)[a]	eNOS IC_{50} (μM)[a]	iNOS IC_{50} (μM)[a]	eNOS/nNOS	iNOS/nNOS
4	S	*N*-(1-(3-(dimethylamino)propyl)-	0.47	12.4	95.1	26	199
5	S	*N*-(1-(3-(cyclopropylamino)propyl)-	0.26	8.72	34	34	131
6	S	*N*-(1-(3-morpholinopropyl)-	0.66	6.0	41.6	9	63
7	S	*N*-(1-(3-((1-ethylpyrrolidin-2-yl)methylamino)propyl)-	0.13	6.33	NT[b]	48	NC[c]
8	S	*N*-(1-(3-adamantanaminopropyl)-	0.36	3.15	18	9	50
9	S	*N*-(1-(2-(dimethylamino)ethyl)-	0.17	3.70	15.7	22	93
10	S	*N*-(1-(2-(piperidin-1-yl)ethyl)-	0.17	9.2	4.61	54	27
11	S	*N*-(1-(2-(1-methylpiperidin-2-yl)ethyl)-	0.09	6.4	9.8	71	109
12	S	*N*-(1-(2-(1-methylpyrrolidin-2-yl)ethyl)-	0.07	4.49	14	64	200
(*R*)-12	S	*N*-(1-(2-(1-methylpyrrolidin-2-yl)ethyl)-	0.06	3.37	22	56	367
(*S*)-12	S	*N*-(1-(2-(1-methylpyrrolidin-2-yl)ethyl)-	0.02	1.92	17	96	850
13	O	*N*-(1-(2-(1-methylpyrrolidin-2-yl)ethyl)-	0.25	13.9	32.7	56	131
14	S	*N*-(1-(1-methylazepan-4-yl)-	0.12	11.5	7.6	94	62
15	O	*N*-(1-(1-methylazepan-4-yl)-	0.43	38.5	49	89	113
(−)-15	O	*N*-(1-(1-methylazepan-4-yl)-	0.40	24.5	20	60	49
(+)-15	O	*N*-(1-(1-methylazepan-4-yl)-	0.56	28.5	73	50	128
16	S	*N*-(1-(8-methyl-8-azabicyclo[3.2.1]octan-3-yl)-	0.76	18.9	11	25	14
17	S	*N*-(1-(quinuclidin-3-yl)-	0.36	12.1	29	33	79
18	S	*N*-(1-(1-methylpiperidin-4-yl)-	0.41	15.8	15	38	36

[a] In a radiometric method, inhibitory activities were measured by the conversion of [^{3}H]-L-arginine into [^{3}H]-L-citrulline.
[b] NT, not tested.
[c] NC, not calculable.

tency for nNOS (compound **(*S*)-12**). The most potent and selective human nNOS inhibitor from the series compound **(*S*)-12** has been selected for further evaluation in a variety of in vivo pain models and the results will be communicated in due course.

Acknowledgments

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17. Enantiomeric separation of **(*R*)-12** and **(*S*)-12** was achieved using chiracel OD–H column (20 × 250 mm) by isocratic elution with ethanol: hexane (contains 0.1% DEA), 8:92 (flow rate: 5.6 mL/min, wavelength: 230 nm).
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19. Co-injections were performed using analytical AD-H column by isocratic elution with IPA: hexanes (contains 0.1% DEA), 15:85 (flow rate: 1 mL/min, wavelength: 254 nm).
20. Enantiomeric separation of (−)-**15** and (+)-**15** was achieved by chiral HPLC separation using chiralpak AD-H column (5 × 50 cm) by isocratic elution with 100% AcCN containing 0.1% DEA (flow rate: 300 mL/min, wavelength: 300 nm). (−)-**15**: $[\alpha]_D = -6.87$ ($c = 0.5$, MeOH); (+)-**15**: $[\alpha]_D = +7.08$ ($c = 0.5$, MeOH).
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26. The free base in anhydrous methanol was treated with HCl (1 M solution in diethyl ether) and stirred at room temperature for 10 min. The solvent was evaporated and dried under reduced pressure to obtain the dihydrochloride salt (trihydrochloride salt for **7**) as a solid. The di- and trihydrochloride salt formation is based on the number of basic amines on the molecule, which was supported by the ^{1}H NMR data in DMSO-d_6, followed by D_2O exchange.
27. Recombinant human iNOS, eNOS and nNOS were produced in Baculovirus-infected Sf9 cells (ALEXIS). In a radiometric method, inhibitory activities were measured by the conversion of [^{3}H]-L-arginine into [^{3}H]-L-citrulline. The enzymatic reaction was carried out in the presence or absence of varying concentrations of the inhibitor in water. The negatively charged [^{3}H]-L-citrulline was separated from the positively charged [^{3}H]-L-arginine using resin beads. Inhibition of enzyme activity by the inhibitor is measured by dividing the enzymatic conversion in the presence of inhibitor divided by the enzymatic conversion in the absence of inhibitor. IC_{50} value is the concentration of compound that gives rise to 50% inhibition. All assays were performed in duplicate.