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A facile synthesis, anti-inflammatory and analgesic activity of isoxazolyl-2,3-dihydrospiro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones

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ABSTRACT

A new series of isoxazolyl-2,3-dihydrospiro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones (**14**) were synthesized by reaction of 4-amino-3-methyl-5-styrylisoxazole **10** with chloroacetic acid followed by a three component reaction with substituted isatins **12** and 1,4-naphthoquinone **13** using Ceric ammonium nitrate (CAN) catalyst under aerial oxidation condition. Structures of these compounds were established on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The title compounds **14a–j** were evaluated for their anti-inflammatory and analgesic activity. Compounds **14d**, **14e** and **14f** exhibited potent anti-inflammatory and analgesic activity as that of standard drugs.

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Exploring novel pharmacological agents with minimum number of synthetic steps and in less time is a major challenge to the chemists. Besides, chemists are facing another challenge for the past two decades, namely, that of developing new transformations that are not only efficient, selective, and high yielding but also environmentally benign.

The spirooxindole system is the core structure of many pharmacological agents and natural alkaloids.[1–3] Naturally occurring spirooxindole alkaloids (Fig. 1), such as spirotryprostatin A (**1**), a natural product isolated from the fermentation of broth *Aspergillus fumigates*, has been identified as a novel inhibitor of microtubule assembly.[4] Alstonisine (**2**) is a useful bioactive natural product. Mitraphylline (**3**) isolated from *Uncaria tomentosa* possesses anti-tumor activity against human brain cancer cell lines.[5] Horsfiline (**4**)[6–11] isolated from *Horsfieldia superba* and elacomine (**5**)[12] isolated from *Eleagnus commutata* find use as indigenous medicine.

Various spiro ring systems have been reported in spirooxindole natural products, for example, alstonisine (**2**) fused with pyrrolidine[13] and tabernoxidine (**6**) fused with piperidine.[14] Spirocyclic isoxazoles such as aerothionin (**7**), aerophobin-1 (**8**), and zamamistatin (**9**) are biologically active alkaloids.[15] The synthesis of spirooxindoles *via* cycloaddition of azomethine ylides to naphthoquinones was reported in the literature.[16] The development of new methods for the synthesis of spirooxindoles is still required due to the immense interest of this system from synthetic and biological standpoints. As a part of our ongoing research in development of new biologically active isoxazole derivatives[17] from readily available starting materials, we envisaged a multi-component reaction for the synthesis of title compounds based on the biological activity of spirooxindole moiety. Herein, we report the synthesis of isoxazolyl-2,3-dihydrospiro [benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones from 2-(3-methyl-5-styrylisoxazol-4-ylamino) acetic acids and their anti-inflammatory and analgesic activities.

The reaction of substituted 4-amino-3-methyl-5-styrylisoxazole **10** with chloroacetic acid in dichloromethane (DCM), led to the formation of 2-(3-methyl-5-styrylisoxazol-4-ylamino)acetic acids **11**, in 65–78% yields.[18] The IR spectra of **11a–g** showed absorption bands around 1700–1720 cm^{-1} and 3250–3270 cm^{-1} for carboxyl group, and 3315–3350 cm^{-1} for amino group. ^{1}H NMR spectra of compounds **11a–g** exhibited three singlets at δ 4.11, 8.35 and 10.73 due to methylene, NH and OH protons respectively. ^{13}C NMR spectra of **11a–g** showed a peak at δ 48.32 due to methylene carbon. Data from the elemental analyses and ESI mass spectra further confirmed the assigned structures of **11a–g**. The three component one-pot reaction was first explored by interaction of 2-(3-methyl-5-styrylisoxazol-4-ylamino)acetic acid **11**, with isatin **12** and 1,4-naphthoquinone **13** in presence of 10 mol % of CAN at ambient temperature in ethanol (15 mL) for 30 min under aerial oxidation condition. The reaction afforded isoxazolyl-2,3-dihydrospi-

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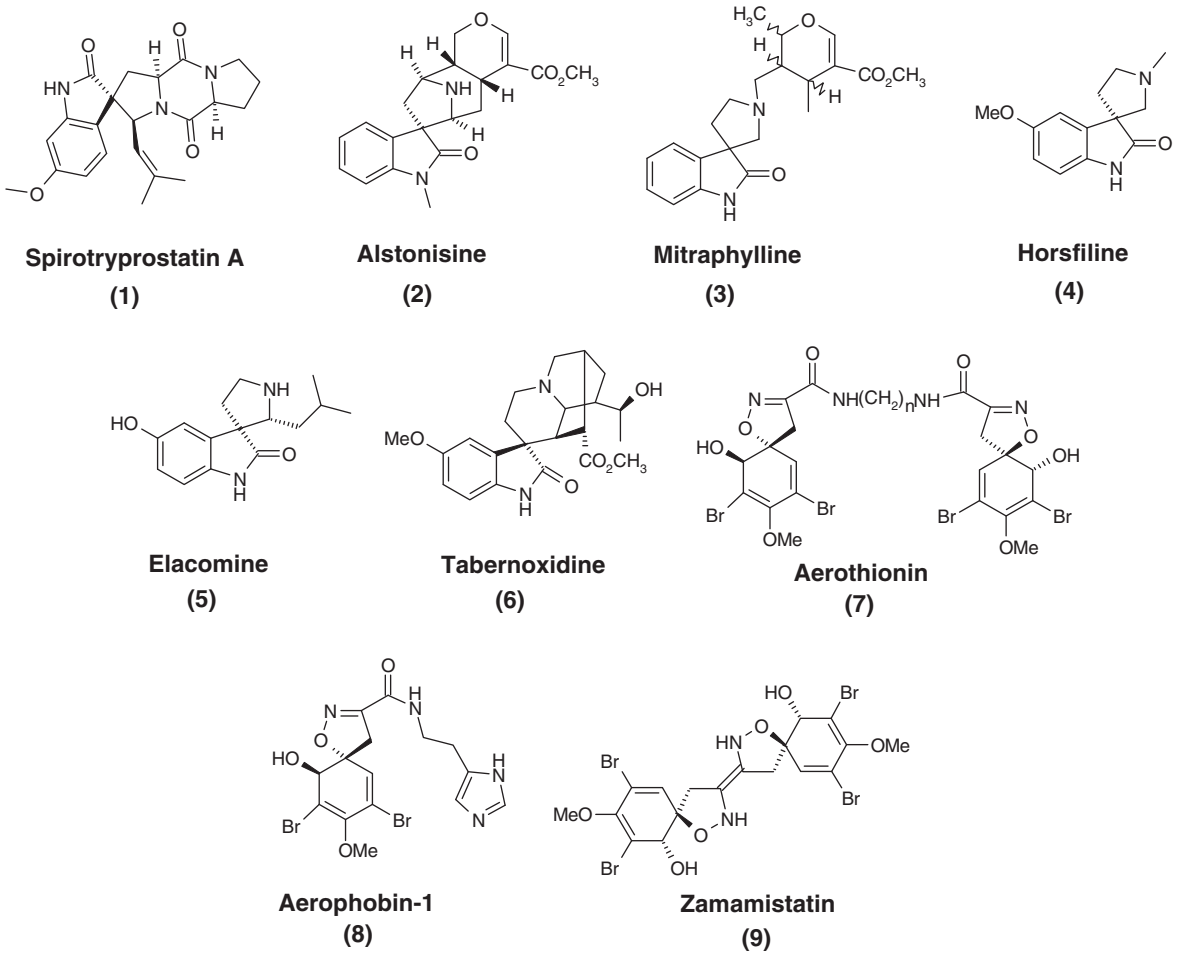


Figure 1. Structures of biologically active spirooxindole alkaloids and spirocyclic isoxazoles as design templates.

ro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-trione **14a** in excellent yield.[19] When the same reaction was carried out in the absence of CAN, the reaction required refluxing condition, longer reaction time, and the product yield is moderate. Hence, CAN is essential for the enhancement of the reaction rate, and to increase the product yield. It has been found that this three-component process works well for any tested combination of 2-(3-methyl-5-styrylisoxazol-4-ylamino)acetic acids, substituted isatins and 1,4-naphthoquinone in ethanol using CAN as catalyst under aerial oxidation condition. By adopting similar procedure, ten new derivatives of **14a–j** have been synthesized by a one-pot three-component reaction. The IR spectra of **14a–j** showed absorption bands around 1685, 1645 cm^{-1} due to carbonyl functional groups and 3265 cm^{-1} due to amino functional group. The ^1H NMR spectra of title compounds **14a–j** displayed two singlets at δ 4.16 and 8.04 due to pyrrolidine methylene protons and NH protons respectively, where as aromatic protons resonated between δ 7.01 and 7.53 as multiplet. In ^{13}C NMR, the peak at δ 79.86 ppm corresponds to the spiro carbon and the peak at δ 168.43 belongs to amide carbonyl carbon. The peaks at δ 183.53 and 183.59 confirmed the presence of quinone carbonyl carbons. Data from the elemental analyses and ESI mass spectra further confirmed the assigned structures of **14a–j**. The structure of the products was elucidated with the help of IR, ^1H NMR, ^{13}C NMR and mass spectral data (Scheme 1).

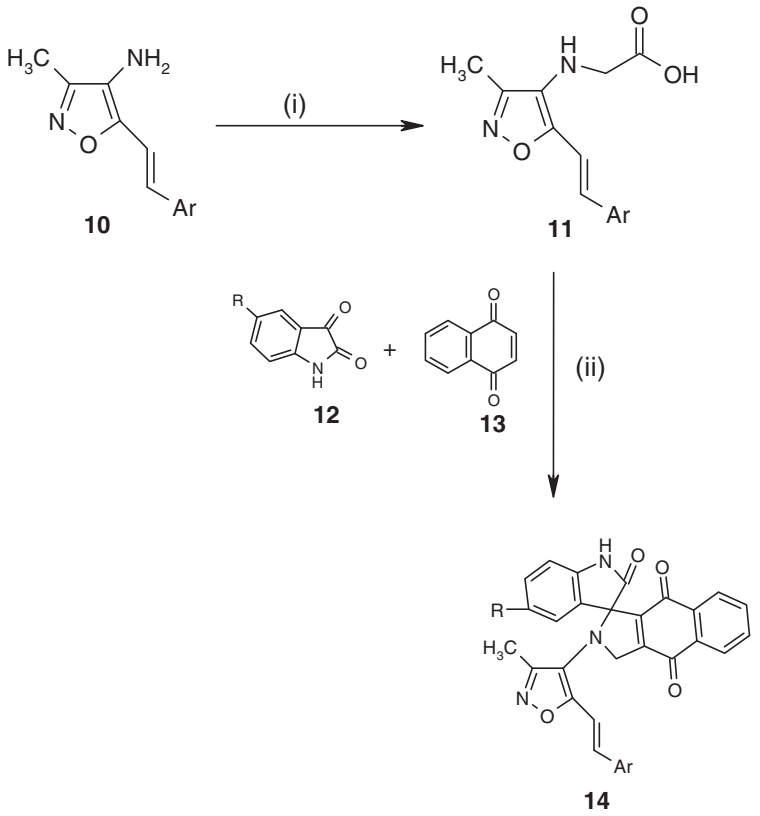
The plausible mechanism involves the 1,3-dipolar cycloaddition of azomethine ylide **A**, generated in situ via decarboxylative condensation of isatin **12** with isoxazole acetic acid **11** to 1,4-naphthoquinone **13** activated by CAN, followed by dehydrogenation under aerial oxidation condition affords the title compound **14**. Ceric ammonium nitrate (CAN) may be activating the double bond of quinone ring by forming a complex, thereby the reaction is facilitated and also it enhances the reaction rate (Scheme 2).

Anti-inflammatory analyses

Anti-inflammatory activity was determined by carrageenan induced paw edema method.[20] Wistar rats of either sex weighing 150–200 g were divided into 6 groups ($n = 6$) and they were fasted 18 h before the experiment with water ad libitum. Group-I received 1% sodium CMC (negative control), Group-II received ibuprofen at a dose of 100 mg/kg (positive control) and Group-III to VI were given the compounds **14a–j** (100 mg/kg). All the compounds **14a–j** were given in oral route. After 30 min, 0.1 mL of 1% carrageenan suspension in normal saline was injected into the subplantar region of the left hind paw of each rat to induce edema. The edema volumes of the injected paw measured with the help of plethysmograph at the interval of 0, 1, 2, 4 and 6 h. The difference between the paw volumes of treated animals were compared with that of the control group and the mean edema volume was calculated. Percentage inhibition was calculated as per the formula, %inhibition = $[(Vo - Vt)/Vo] \times 100$, where Vo = volume of the paw control at time t, Vt = volume of the paw of drug treated at time t.

Analgesic analyses

The analgesic activity was determined by acetic acid induced writhing.[21] Swiss albino mice ($n = 6$) of either sex selected by



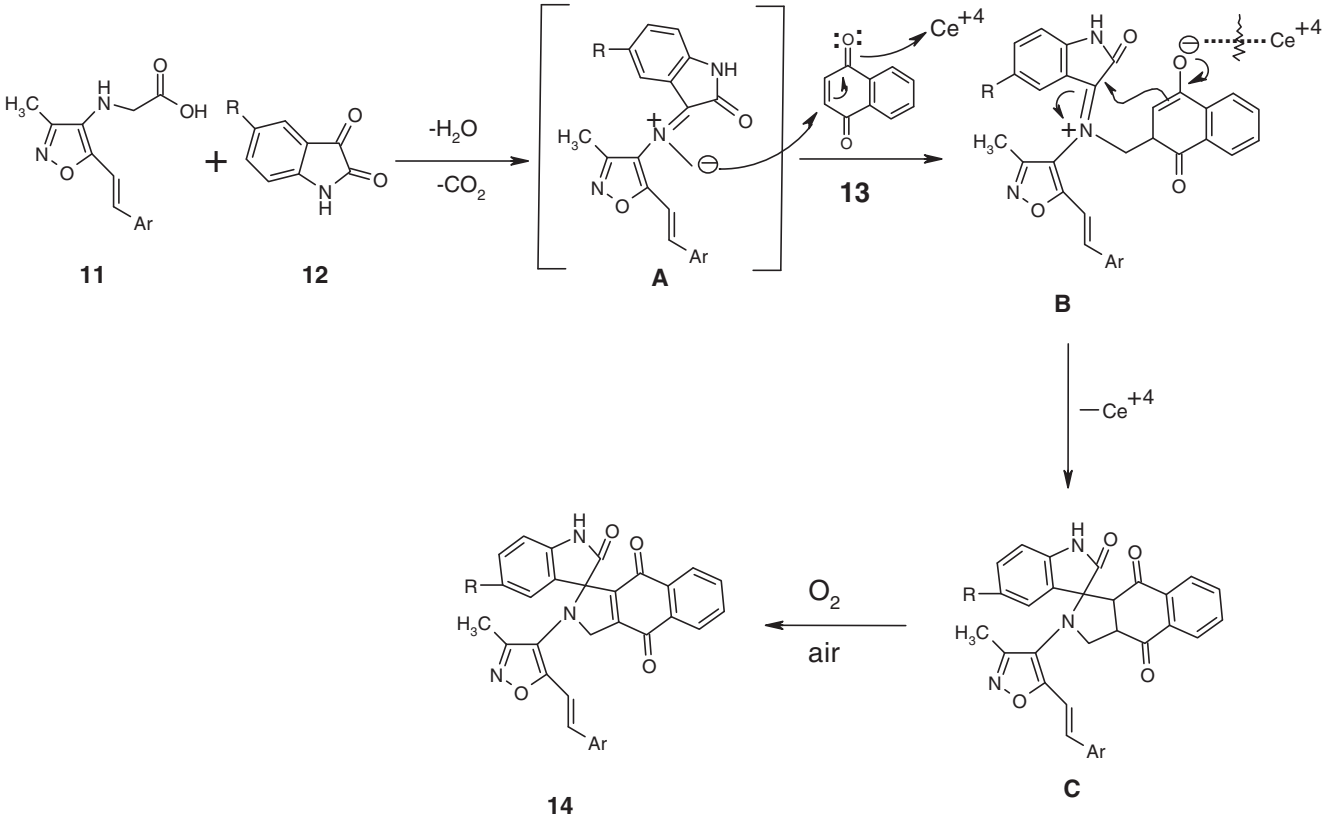
11a,	Ar = C_6H_5	**14a,** Ar = C_6H_5	R = H
11b,	Ar = 4-$CH_3C_6H_4$	**14b,** Ar = 4-$CH_3C_6H_4$	R = H
11c,	Ar = 4-$CH_3OC_6H_4$	**14c,** Ar = 4-$CH_3OC_6H_4$	R = H
11d,	Ar = 4-ClC_6H_4	**14d,** Ar = 4-ClC_6H_4	R = H
11e,	Ar = 2,4-$Cl_2C_6H_3$	**14e,** Ar = 2,4-$Cl_2C_6H_3$	R = H
11f,	Ar = 2,6-$Cl_2C_6H_3$	**14f,** Ar = 2,6-$Cl_2C_6H_3$	R = H
11g,	Ar = 2-OHC_6H_4	**14g,** Ar = 2-OHC_6H_4	R = H
		14h, Ar = C_6H_5	R = Cl
		14i, Ar = C_6H_5	R = OCH_3
		14j, Ar = C_6H_5	R = CH_3

Scheme 1. Synthesis of isoxazolyl-2,3-dihydrospiro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones (**14a–j**). Reagents and conditions: (i) chloroacetic acid (1 mmol), dichloromethane, 0 °C, stirring, 2 h; (ii) ethanol, CAN (10 mol %), rt, stirring, 30 min, O_2 (air).

random sampling technique were used for the study and divided into 6 groups. The animals were fasted 18 h before the experiment with water ad libitum. Diclofenac (50 mg/kg) was administered as standard drug for comparison. The test compounds **14a–j** (50 mg/kg) were administered orally 30 min. after the administration of compounds. All the mice were given 1.0% v/v solution of acetic acid, ip the dose being 1 mL/100 g of the mice and the writhings produced in these animals were counted for 20 min. The no. of writhings produced in the treated groups was compared with those in the control group. Percentage inhibition was calculated as per the formula, % inhibition = [(Average writhes in control − Average writhes in test)/Average writhes in control] × 100.

The anti-inflammatory activity of the title compounds, **14a–j** were evaluated by carrageenan-induced paw edema method in rats[20] at a dose of 100 mg/kg body weight using ibuprofen as a reference drug. Results were expressed as a mean ± S.E. The anti-inflammatory properties were recorded at successive intervals of 0, 1, 2, 4 and 6 h and compared with that of standard ibuprofen. The anti-inflammatory activity data (Table 1) indicated that all the compounds **14a–j** exhibited significant activity by decreasing the paw volume that was produced by carrageenan. Among all the compounds tested, it is interesting to note that the compounds **14d, 14e** and **14f** showed better anti-inflammatory activity, may be due to the presence of chloro substitution on the benzene ring. The presence of electron donating methyl and methoxy groups on benzene ring (**14i** and **14j**) did not influence the activity much.

The analgesic activity of the newly synthesized compounds **14a–j** were determined in vivo by acetic acid induced writhing method[21] in mice at a dose of 50 mg/kg body weight. All the compounds **14a–j** produced significant activity when compared to standard diclofenac. Compounds **14d, 14e** and **14f** showed more significant activity compared to other test compounds (Table 2). These compounds **14d, 14e** and **14f** decreased the number of writhings produced in mice to a remarkable extent. This may be due to the presence of chloro substituent in these compounds.



Scheme 2. Plausible mechanism for the formation of isoxazolyl-2,3-dihydrospiro[benzo[*f*] isoindole-1,3′-indoline]-2′,4,9-triones

Table 1
Anti-inflammatory activity of isoxazolyl-2,3-dihydrospiro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones[a] (**14a–j**)

Group[b]	Paw volume (mL of Hg)[c]				
	0 h	1 h	2 h	4 h	6 h
14a	0.37 ± 0.01	0.75 ± 0.03	0.84 ± 0.08^{ns}	0.75 ± 0.06**	0.60 ± 0.03**
14b	0.34 ± 0.03	0.70 ± 0.05	0.80 ± 0.05^{ns}	0.69 ± 0.03**	0.54 ± 0.01**
14c	0.36 ± 0.03	0.80 ± 0.01	0.91 ± 0.04^{ns}	0.65 ± 0.02**	0.60 ± 0.03**
14d	0.27 ± 0.04	0.54 ± 0.1	0.69 ± 0.03	0.65 ± 0.03**	0.41 ± 0.05***
14e	0.25 ± 0.08	0.57 ± 0.06	0.71 ± 0.03	0.54 ± 0.02	0.32 ± 0.01***
14f	0.22 ± 0.02	0.54 ± 0.08	0.70 ± 0.03	0.67 ± 0.03*	0.40 ± 0.03***
14g	0.31 ± 0.03	0.78 ± 0.02	0.81 ± 0.03^{ns}	0.80 ± 0.04*	0.52 ± 0.02**
14h	0.40 ± 0.01	0.82 ± 0.04	0.83 ± 0.02^{ns}	0.82 ± 0.03**	0.63 ± 0.03**
14i	0.34 ± 0.04	0.73 ± 0.01	0.91 ± 0.05^{ns}	0.81 ± 0.03**	0.60 ± 0.01**
14j	0.33 ± 0.08	0.77 ± 0.02	0.89 ± 0.03^{ns}	0.83 ± 0.01**	0.55 ± 0.05**
Control	0.36 ± 0.3	0.90 ± 0.05	1.07 ± 0.08	1.2 ± 0.05	0.96 ± 0.03
Ibuprofen	0.30 ± 0.5	0.66 ± 0.03*	0.70 ± 0.05***	0.60 ± 0.05***	0.40 ± 0.05***

Statistically significant compound to respective control value $^{*}P$ <0. 05, $^{**}P$ <0.01, $^{***}P$ <0.001.
^{ns}Nonsignificant, compared to control.
[a] n = 6, number of animals used in each group.
[b] Dose levels: test compound (100 mg/kg b.wt) Ibuprofen (100 mg/kg b.wt).
[c] Values are expressed as mean ± S.E.

Table 2
Analgesic activity of isoxazolyl-2,3-dihydrospiro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones (**14a–j**)

Animal group[a]	No. of writhings[b]	% inhibition
14a	30 ± 1.31*	22.43 ± 1.41
14b	34 ± 1.50*	26.27 ± 2.00
14c	31.1 ± 1.36*	19.58 ± 1.60
14d	21.5 ± 2.40***	50.41 ± 1.31
14e	20.4 ± 1.54***	44.80 ± 2.15
14f	22.88 ± 1.50***	48.30 ± 1.50
14g	33.61 ± 1.48*	20.40 ± 1.50
14h	34.61 ± 1.83*	16.51 ± 1.50
14i	27 ± 1.38*	18.42 ± 2.05
14j	29 ± 1.94*	19.50 ± 1.80
Control	41 ± 1.25	—
Diclofenac	19 ± 1.23***	53.65 ± 1.92

Statistically significant compound to respective control value $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001.
[a] Dose levels: test compound (50 mg/kg b.wt) Diclofenac (50 mg/kg).
[b] Values are expressed as mean ± S.E.

Rests of the compounds (**14a**, **14b**, **14c**, **14g**, **14h**, **14i** and **14j**) are moderately active.

In conclusion, we reported a simple and efficient multi-component protocol for the synthesis of isoxazolyl-2,3-dihydrospiro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones, using inexpensive and commercially available materials with potent medicinal properties. The newly synthesized novel isoxazolyl-2,3-dihydrospiro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones **14a–j** were evaluated for their anti-inflammatory and analgesic activity. Compounds **14d, 14e** and **14f** are proved to possess remarkable anti-inflammatory and analgesic activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.053.

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18. *General procedure for the synthesis of 2-(3-methyl-5-styrylisoxazol-4-ylamino)acetic acids (**11a–g**):* To a vigorously stirred solution of 4-amio-3-methyl-5-styrylisoxazole **10a** (1 mmol) in dichloro methane (DCM) (15 mL) at 0 °C, was added chloroacetic acid (1 mmol) in dichloro methane (5 mL) and stirring continued for 2 h. The resulting reaction mixture was washed with water (3 × 10 mL) and extracted with dichloro methane (40 mL). The combined layers were dried over anhyd. Na_2SO_4 and filtered and further dried over vacuo. The crude product was purified by recrystallization from ethyl acetate.
19. *General procedure for the synthesis of isoxazolyl-2,3-dihydrospiro[benzo[f]isoindole-1,3′-indoline]-2′,4,9-triones (**14a–j**):* A mixture of 2-(3-methyl-5-styrylisoxazol-4-ylamino)acetic acid **11a** (1 mmol), isatin **12a** (1 mmol), 1,4-napthoquinone **13** (1 mmol) and CAN (10 mol %) was stirred in ethanol(15 mL) for 30 min under aerial oxidation condition. Progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured onto crushed ice and the separated solid product was filtered and purified by recrystallization from ethyl acetate to get isoxazolyl-2,3-dihydrospiro[benzo[*f*]isoindole-1,3′-indoline]-2′,4,9-triones **14a–j**.
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