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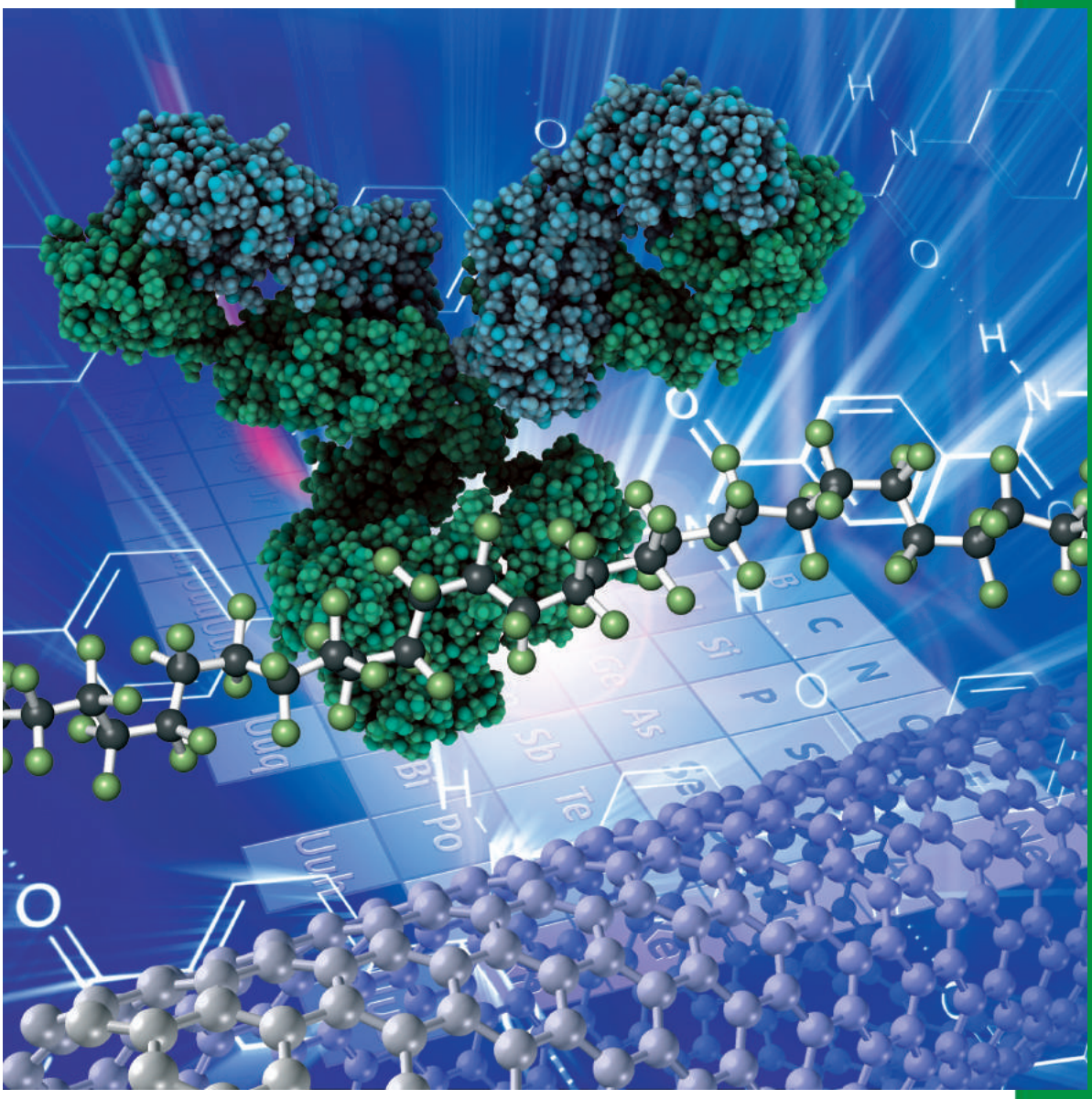

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Medicinal Chemistry & Drug Discovery

Design, Synthesis and Anticancer Evaluation of Spiro [cyclohexane-1,1′-indene]-2,5-diene Analogues

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A series of spirocyclohexaneindene-2,5-diene derivatives were synthesized from Spiro-Acid **5** which in turn was prepared from the easily accessible starting materials. The structure of spiro-ester **4** was thoroughly further confirmed by 2D NMR analysis. The synthesized compounds were screened for anticancer activity using murine melanoma cell line (B16F10), human breast cancer cell line (MCF-7) and human non-small cell lung carcinoma cell lines (A549). Among them **7f** (tri fluorobenzene) and **7g** (di fluorobenzene) analogues were the most active compounds in all three cell lines in the series.

Introduction

Spirodienones are molecules containing two rings with one shared atom, occurring widely in nature with potential biological activities and are considered important in biosynthetic pathways. The Spiro design is becoming more ubiquitous as outlined in drug discovery, and has been the subject of recent progress on the new synthetic routes to facilitate incorporation of Spiro scaffolds into more pharmaceutically active molecules.[1] Spiro rings of different sizes convey both increased three dimensionality for potential improved activity, and novelty for patenting purposes. Due to the structural stringency, these compounds are one of the less focused classes in recent times. However, the chemistry of Spirodienones has attracted considerable interest over the years due to their presence in diverse natural products and biologically active molecules, such as Anti-HBV, anti-proliferative and anti-inflammatory (Figure 1).[2–8] Moreover, their integral characteristic with the Spiro cyclic skeleton and the enone functionality renders them to be a class of synthetically useful building blocks in organic synthesis. Thus, there is great interest in the development of practical methods for their prompt assemblage from simple precursors. The new Spiro natural products are still being isolated and synthesized, total syntheses of some compounds isolated many years ago were achieved using the fewer refined methodologies available at the time also been improvised by applying modern procedures and methodologies. However, due to the distinctive properties of Spirodienones, especially the relatively congested quaternary carbon centre, it has been a challenging task for chemists to develop a synthetically applicable methodology. Hence, highly efficient, mild, and streamlined synthetic routes are still in great demand to construct these substrates.[9]

Herein, we disclose the successful execution of our design, which leads to a short and robust method for constructing the Spiro indene cyclohexadienone.

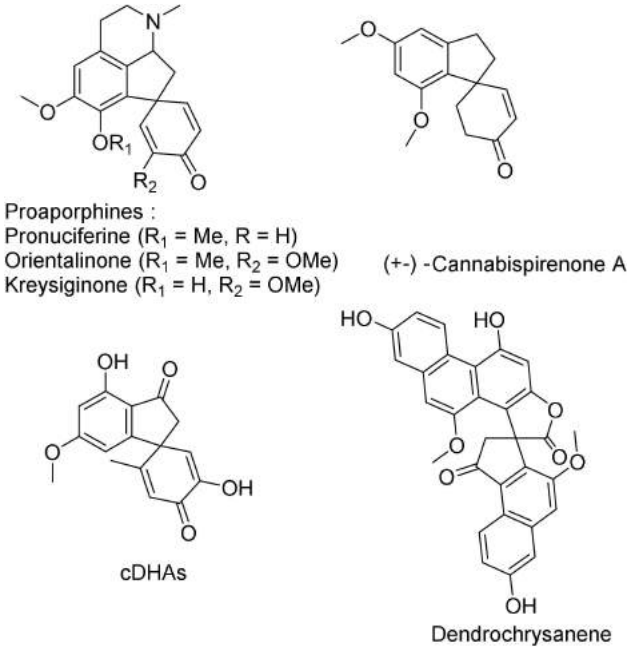

Figure 1. Representative Examples of related natural occurring spirodiene cyclohexdienones

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Results and discussion

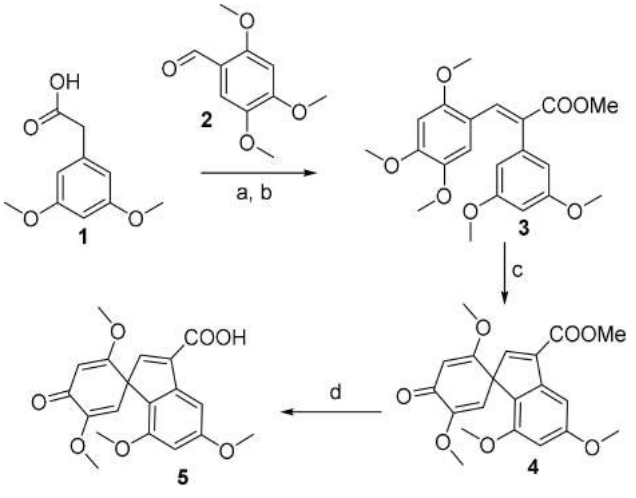
Chemistry

Synthesis of key building block **5** commenced with inexpensive and readily available starting materials 2-(3,5-dimethoxyphenyl) acetic acid **1** and 2,4,5-trimethoxybenzaldehyde **2**. Perkin condensation was carried out with **1** and **2** in the presence of acetic anhydride and Et_3N at elevated temperature for 24 h provided ester **3** in good yields.[10] A fewer bases like Piperidine, DIPEA and K_2CO_3 were screened to improvise the yield, however Et_3N was found to be superior over the other bases. Then, the ester **3** was subjected to the key step of constructing Spiro indene cyclohexadienone **4** under Lewis acid mediated conditions. In order to determine the optimal conditions for synthesis of Spiro-ester **4**, the several Lewis acids were screened with or without addition of oxidizing agents (**Table 1**).[10–13] We have observed best results using PIFA and

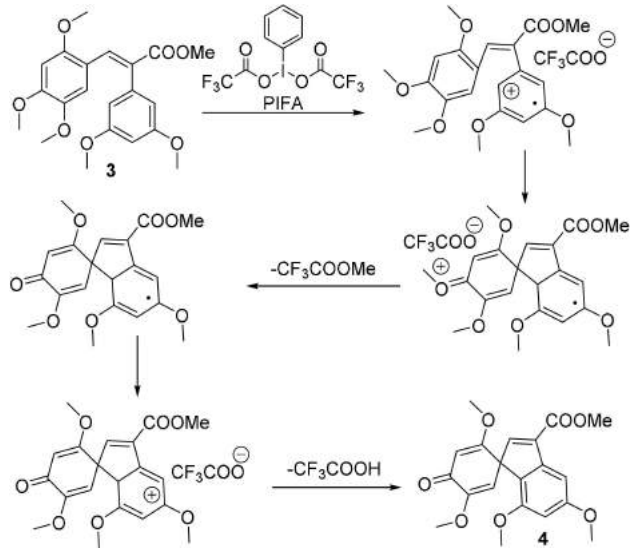
Table 1. Attempted conditions for conversion from **3** to **4**

S.No	Reaction Conditions	Temperature and Time	Yield
1	$FeCl_3$, CH_2Cl_2	-40 °C - rt, 1 h	35%
2	CAN, $NaHCO_3$, CH_3CN	rt, 6 h	23%
3	MnO_2, TFA	0 °C - rt, 3 h	29%
4	$NaNO_2$, TFA	0 °C - rt, 3 h	32%
5	MnO_2, BF_3-Et_2O, CH_2Cl_2	rt, 8 h	26%
6	CH_3SO_3H/DDQ, CH_2Cl_2	0 °C - rt, 2 h	Traces
7	PIDA/BF_3-Et_2O, CH_2Cl_2	0 °C - rt, 2 h	35%
8	PIFA/BF_3-Et_2O, CH_2Cl_2	0 °C - rt, 2 h	41%
9	PIFA/BF_3-Et_2O, CH_2Cl_2	-78 °C - 0 °C, 2 h	70%

BF_3.Et_2O at −78 °C to 0 °C for 2 h with 70% yield. The structure of Spiro-ester **4** was further re-confirmed by 2D ^{1}HNMR experiments viz., HSQC and HMBC (refer SI). The plausible mechanism[14] for the conversion of open chain ester **3** to Spiro-ester **4** is depicted in **Scheme 2**. Hydrolysis of Spiro-ester **4**

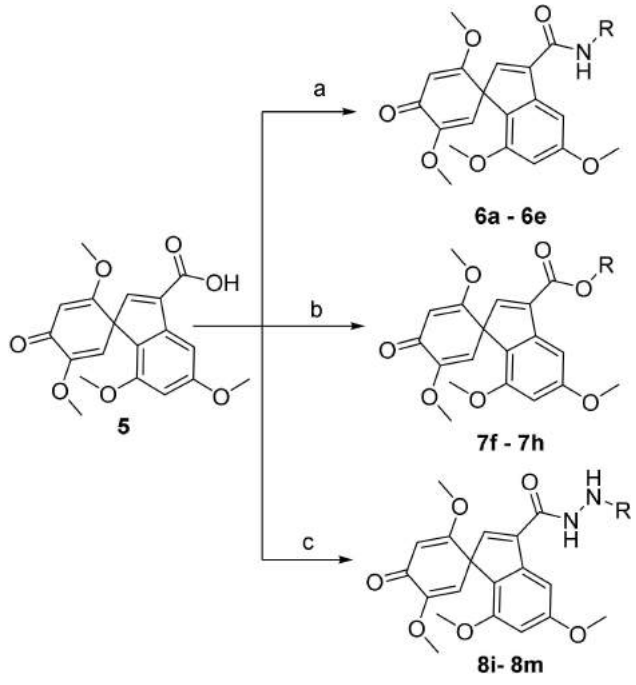


Scheme 1. Synthesis of key intermediate **5**
a) **2**, TEA, $(Ac)_2O$, 120 °C, 24 h, b) H_2SO_4, MeOH, 65 °C, 16 h, 56%; c) PIFA, BF_3.Et_2O, DCM, −78 °C - 0 °C, 2 h, 70%; d) 2 N NaOH, MeOH, 65 °C, 18 h, 80%



Scheme 2. Plausible SET mechanism for conversion from **3** to **4**

using 2 N NaOH in methanol at 65 °C for 18 h gave key intermediate Spiro-acid **5** in 80% yield (**Scheme 1**). Then, the precursor acid **5** was further derivatized to prepare NCE's for activity screenings. In this regard, **5** was converted its corresponding amides (**6a - 6e**) under HATU, DIPEA, rt, 16 h with 80–90% yields (**Scheme 3**). EEDQ, THF, 65 °C for 2 h



Scheme 3. Synthesis of Amides, Esters and Hydrazides
a) Corresponding Amine, HATU, DIPEA, THF, rt, 6 h, 63–83%. b) Corresponding Alcohol, EEDQ, THF, 65 °C, 2 h; 70–89%. c) Corresponding Hydrazine, HATU, DIPEA, THF, rt, 16 h, 66–81%.

conditions[15] provided better results with quick conversion to afford the corresponding esters (**7f – 7h**) of **5** over other reagents like HATU, EDC/DMAP, DCC/DMAP at rt to heating conditions. Hydrazides (**8i – 8m**) were successfully synthesized under HATU, DIPEA conditions in 70–80% yields (**Scheme 3**).

Since the anticancer activity of Cannabis spirones was reported in literature,[16–17] we desired to evaluate the prepared Spiro indene cyclohexadiene derivatives (**Figure 2**) against

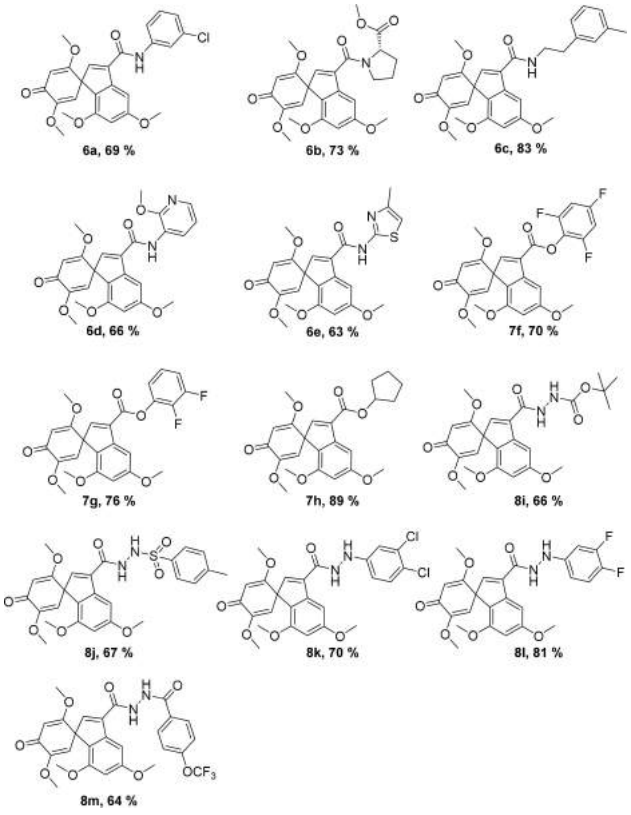


Figure 2. Synthesized Spiroindene-2,5-diene Amides, Esters and Hydrazides

three different cancer cell lines, murine melanoma cell line (B16F10), human breast cancer cell line (MCF 7) and human non-small cell lung carcinoma cell lines at various concentrations and the results were summarized in Figure **3**, **4**, **5** and **6**. Interestingly, the Fluoro substituted aryl esters shown promising Anticancer activity among the other derivatives.

Biology

MTT assay to screen new compounds for anticancer activity:

Cell culture:

The new compounds were screened for anticancer activity using three different cancer cell lines, murine melanoma cell line (B16F10), human breast cancer cell line (MCF-7) and human non-small cell lung carcinoma cell line (A549). B16F10 and MCF-7 cells were cultured in Dulbecco's modified eagle medium and A549 cells in Dulbecco's modified eagle medium F-12 supplemented with fetal bovine serum (5%) and antibiotic (1%). All reagents were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India. The incubation of cells was done in humidified atmosphere and 5% CO_2 at 37 °C.

MTT assay procedure:

In 96 well plate 8×10^3 cells were seeded and incubated overnight. The treatment of cells with novel compounds was done at two different doses (100 μM and 10 μM) for 48 hours. The MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added and after 4 hours, DMSO was added to dissolve purple formazan crystals. The absorbance was measured at 570 and 650 nm by Spectramax (Molecular Devices, USA).[18] Some best active compounds were selected to determine IC_{50} values in three different cancer cell lines such as B16F10 (murine melanoma cell line), MCF-7 (human breast cancer cell line) and A549 (human n**on-Small cell lung carcinoma cell line)** and same procedure was followed as described above. Six compounds were tested at 10 different concentrations such as 200 μM, 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM, 1.562 μM, 0.781 μM and 0.39 μM. The experiment was repeated as described above with another batch of cells

MTT assay is an enzyme based colorimetric assay method which was used to screen all new compounds for their anticancer activity [**Figure 3**]. From the results, it was observed

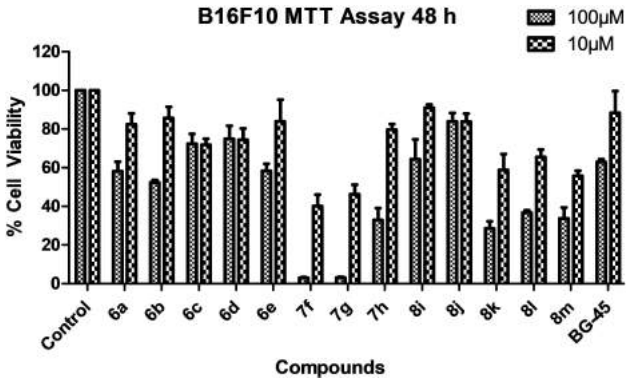


Figure 3. Anticancer activity of all novel compounds from Spiro series by MTT assay. Compounds were tested for cytotoxicity on B16F10 cells (Murine melanoma cell line) for 48 h.

that good anticancer activity was possessed by compounds at 100 μM concentration whereas moderate activity was showed by few compounds at 10 μM. We have done our initial screening with all thirteen synthesized novel compounds to determine if they could exhibit anti-proliferative effect in B16F10 cell lines. There are six compounds coded as **7f**, **7g**, **7h**, **8k**, **8l** and **8m** were found to be more promising. In our next study we have tested those 6 most active compounds in wider range of concentrations to determine their precise 50% inhibition concentration (IC50). We have performed the dose response experiment with two other cancer cell lines and

similar trend of anti-proliferative potency of the selected compounds has been observed [**Figure 4, 5 and 6**]. It has been

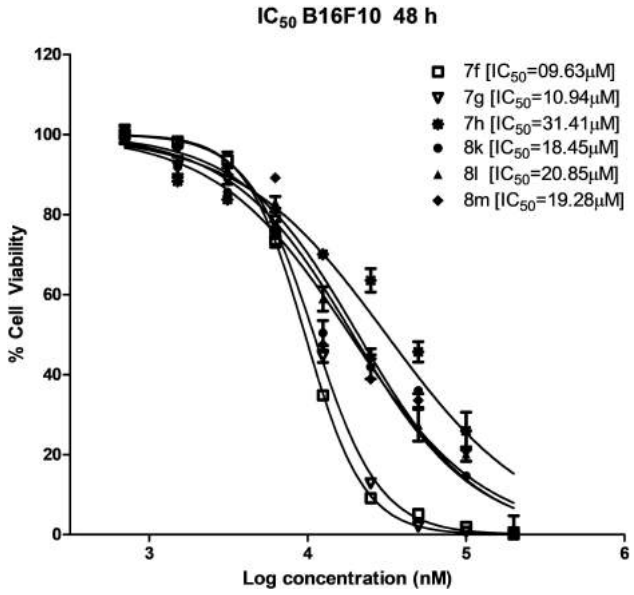


Figure 4. IC_{50} values of analogues against B16F10 cell line

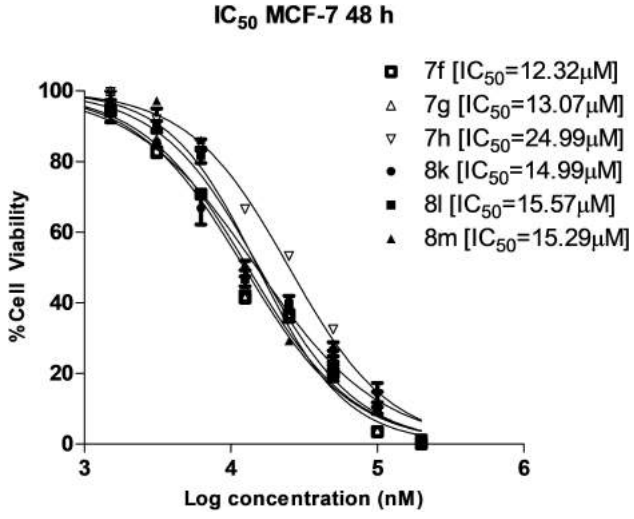


Figure 5. IC_{50} values of analogues against MCF-7 cell line

found that compound **7f** and **7g** were the most active compounds in all three cell lines in the series

The promising compounds were screened further to find out their IC_{50} values. **7f**, **7g**, **7h**, **8k**, **8l** and **8m** were evaluated in ten different doses on B16F10, MCF-7 and A549 cell lines for 48 hours and cell viability was measured using MTT assay. Compounds found to show potent anticancer activity having IC_{50} in range of 9 μM −32 μM.

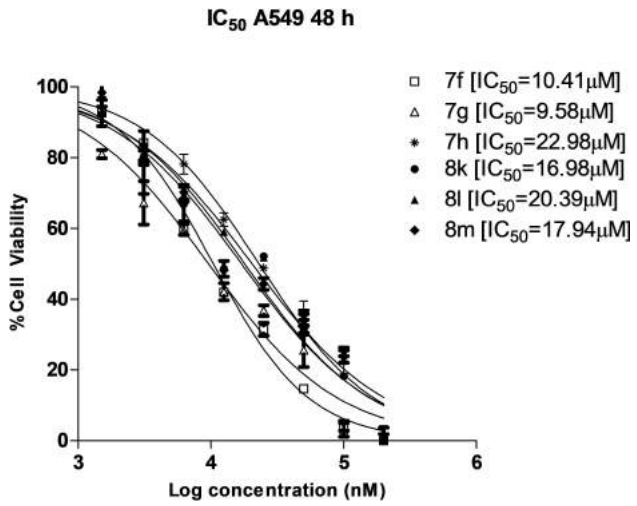


Figure 6. IC_{50} values of analogues against A549 cell line

Conclusions

In conclusion, we have developed a short and efficient route for the construction of relatively challenging Spiro indene dienone acid via a key step involving Lewis acid in the formation of Spiro skeleton. Further obtained key intermediate Spiro-acid was derivatized to various novel amides, esters and hydrazides and tested against anticancer activity using murine melanoma cell line (B16F10), human breast cancer cell line (MCF-7) and human non-small cell lung carcinoma cell lines (A549). Among them **7f** (tri fluorobenzene) and **7g** (di fluorobenzene) analogues were found to be the most active with IC50 value of 9.63 μM and 13.07 μM respectively. We believe that these simple and quick accesses to novel Spiro indene dienone ester or acid could open up convenient way for further diversification and to generate the compounds with potential pharmacological interest.

Supporting Information Summary

Detailed information about the experimental procedure, spectral characterization data such as ^{1}H NMR and ^{13}C NMR, FTIR along with HRMS spectra of all synthesized products are provided.

Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Anticancer Evaluation · PIFA · Spiro Cyclization · SET mechanism · Spiro dienes

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