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Antitumor Activity of Selected Derivatives of Pyrazole- Benzenesulfonamides from Dilithiated C(α), N-Phenylhydrazones and Lithiated Methyl 2-(Aminosulfonyl)benzoate

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Abstract

Several pyrazole-benzenesulfonamides were subjected to biological evaluation involving tumor formation on potato discs caused by Agrobacterium tumefaciens. This assay led to some excellent and promising initial results with three of the pyrazole compounds showing increased tumor inhibition when compared to a recognized standard, camptothecin. The select pyrazole-benzenesulfonamides were prepared by condensation-cyclization of several dilithiated C(α),N-phenylhydrazones with lithiated methyl 2-aminosulfonyl-benzoate.

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Several pyrazole-benzenesulfonamides were subjected to biological evaluation involving tumor formation on potato discs caused by Agrobacterium tumefaciens. This assay led to some excellent and promising initial results with three of the pyrazole compounds showing increased tumor inhibition when compared to a recognized standard, camptothecin. The select pyrazole-benzenesulfonamides were prepared by condensation-cyclization of several dilithiated C(α),N-phenylhydrazones with lithiated methyl 2-aminosulfonyl-benzoate.

Introduction

Bioassay methods used in assessing antitumor activity of agricultural plant products have yielded important discoveries of agrochemical materials including camptothecin, vincristine, vinblastine, and podophyllotoxin derivatives [1-5].

The camptothecin compound used in this investigation consists of a single molecular system containing five fused-rings [6]. It is commercially available, or it can be prepared by a multi-step synthon.

Inhibition of *Agrobacterium tumefaciens*-induced tumors in potato discs is an assay based on antimitotic activity [6, 7,] Inhibition of tumor initiation on potato discs and subsequent growth showed a good correlation with agrochemical compounds and extracts active in the 3PS11 leukemic mouse assay [8 -12].

Compounds currently used to treat cancer have been tested in this bioassay and shown to completely inhibit formation of tumors on the potato discs. They do not affect the growth of the bacterium or the transfer of the plasmid into the potato tissue [8-12].

Camptothecin, paclitaxel, podophyllin, vinblastine and vincristine inhibited tumor formation in the potato disc assay [4, 5]. *Agrobacterium tumefaciens* causes Crown Gall disease on woody and herbaceous plants [8, 9]. The potato tumor induction assay uses this bacterium to induce tumors on potato discs. The assay detects compounds that inhibit cell division at any point in the cell cycle.

The overall objective for this second part of the two-part study was to determine the anti-tumor activity of the selected pyrazole-benzene-sulfonamides [pyrazoles 1-6] obtained earlier and shown in Figure 1, and detailed in Table 1. Figure 2 is an illustration for pyazole 3, and an ORTEP diagram (Figure 3) was obtained earlier from a single crystal X-ray analysis for substituted pyrazole 3.

Procedure [1, 10]

The *Agrobacterium tumefaciens* (bacterium) was added to potato discs contained in a 24-well plate. Controls included camptothecin (a positive control) and solvents used, usually water and a minimum amount of alcohol, and the test compounds. After incubation for 14 days, the potato discs were stained with potassium iodide solution. Discs stained dark purple or black whereas the tumors were white to cream colored.

The number of tumors was counted for each test, and the experiment was repeated three times with three replicates per treatment, see Figures 4 and 5. Each substituted pyrazole test chemical (1-6) [1] was tested at 10 ppm for its effect on tumor induction, the growth of the bacterium and the transfer of the plasmid to the potato disc (Table 1).

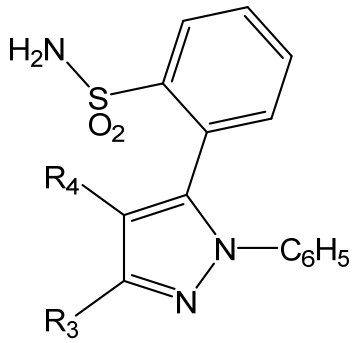


Figure 1. Structural formula for pyrazole-benzenesulfonamides,1-6.

Table 1. Anti-tumor activity of derivatives of 2-(1-phenyl-1*H*-pyrazol-5-yl)-benzenesulfonamides.

Compound	R_3	R_4	% Inhibition
1	3,4,5- $(CH_3O)_3C_6H_2$	H	40
2	3,4- $(CH_3O)_2C_6H_3$	H	45
3	4-$CH_3OC_6H_4$	H	50
4	4-$CH_3C_6H_4$	H	1
5	C_6H_5	H	+26
6	$C_6H_5CH_2$	C_6H_5	6
Camptothecin			26
Control			0

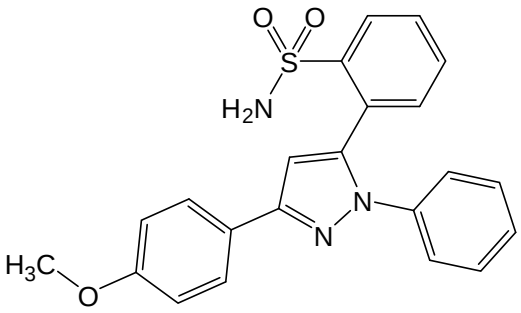


Figure 2. 2-[3-(4-methoxyphenyl)-1-phenyl]-1*H*-pyrazolyl-5-yl]-benzenesulfonamide, 3.

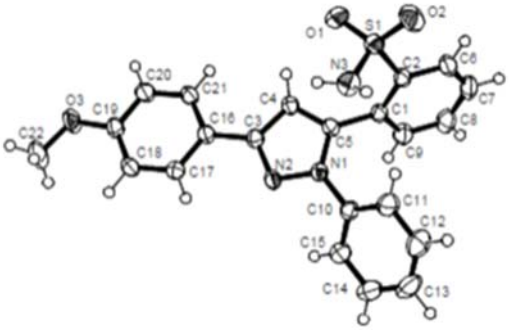


Figure 3. ORTEP diagram for pyrazole/compd. 3.

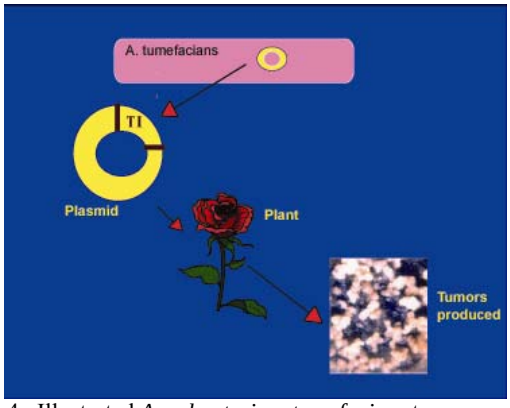


Figure 4. Illustrated *Agrobacterium tumefaciens* tumor assay.

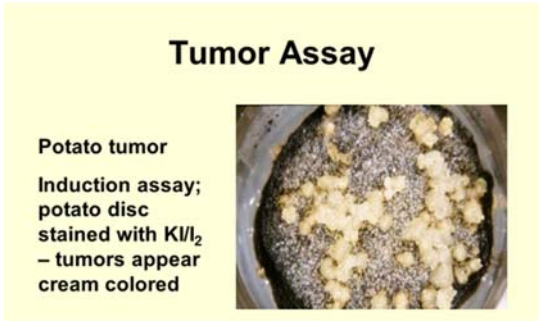


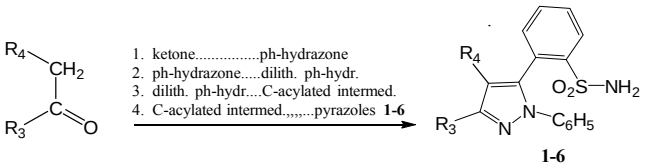
Figure 5. Representative induction assay.

Conclusions

Biological activity ranged from 50% inhibition for pyrazole 3 to +26% stimulation of tumor formation for pyrazole 5. The presence of a methoxy moiety on the phenyl ring R_3 significantly enhanced the inhibitory activity. Pyrazoles 1, 2 and 3 containing methoxyphenyl pendant groups gave the highest, 50% inhibition. The lack of substituents on the R_3 phenyl ring in pyrazole 5 stimulated an increase the number of tumors formed. Pyrazoles 4 and 6 which contained no methoxy groups showed very little inhibition. There was no effect of test chemical pyrazoles on growth of the bacterium or the Ti plasmid transfer.

Substituted pyrazoles 1-6 were prepared, Scheme 1, at the College of Charleston and investigated because of their potential for extensive uses including agricultural biological evaluation at Clemson University, Department of Entomology, Soils and Plant Sciences, as agrochemical agents with antitumor activity for tumors initiated by *Agrobacterium tumefaciens* [11-23] .

The ongoing development of the syntheses and characterizations of 1*H*-pyrazoles and related compounds, begins with easily prepared phenylhydrazones, The entry phenylhydrazones are best dilithiated with excess lithium diisopropylanide (LDA) followed by the condensation of the resulting 1,4-dianion type intermediate(s) with lithiated and/or dilithiated methyl 2-aminosulfonylbenzoate [anion-anion Claisen-type condensation] to afford *C*-acylated intermediates that were not isolated, but were readily cyclized directly with addition of dilute hydrochloric acid to afford the targeted 3,5-unsymmetrucal substituted pyrazole products, 1-6 [24-30].



1-6

Scheme 1: Preparation of Pyrazole-Benzenesulfonamides 1-6; for R_3 and R_4, see Table 1.

Remarks

The three faculty authors (NDC, CFB, CRM) have collaborated in several projects involving undergraduate students generating written reports, presentations at local poster sessions, presentations at Annual Meetings of the South Carolina Academy of Science, and journal publications.

When applicable, the students are usually listed on activities associated with other persons involved with the investigation. The Clemson University students, (JMG, DEL) John Gum and Darby Lyles, performed the biological evaluation assay, but were unable to present their results to the 2008 annual meeting of the SCAS. Instead, Dr. Camper presented the overall project to an exceptionally well attended Topical Session at the meeting.

We had minimal email contact with NDC during the next two years, including the next SCAS meeting. The only indication that we had concerning Dr. Camper's declining health was observing that he had aged considerably since the last SCAS meeting. Several months later we received word of his passing.

In addition to the enthusiastic personal presentation by Dr. Camper to the SCAS, we had no indication of any manuscript being planned, or in preparation, for reporting the introductory antitumor assay for use of pyrazole-benzenesulfonamides 1-6,

Agrobacterium tumefacien, so we elected to prepare and submit a manuscript for publication starting with the information obtained from Dr. Camper's oral presentation slides.

Dedication

This manuscript and its publication is dedicated to memory of N. Dwight Camper, a 43-year faculty member at Clemson University, who also served two terms as President of the South Carolina Academy of Science, in addition many research involvements with undergraduate and graduate students, other faculty members and other professionals.

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