THE ROLE OF HYDROXAMIC ACIDS IN BIOCHEMICAL PROCESSES

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SUMMARY: Hydroxamic acids, a group of naturally occurring and synthetic weak organic acids of general formula RC(=O)N(R')OH, are widespread in the tissues of plants, in metabolites of bacteria and fungi, including complex compounds. Hydroxamic acids and their derivatives fulfill a variety of important roles in biology and medicine; here we provide a comprehensive brief review of the most basic medicinal chemistry and pharmacology of hydroxamate molecules.

Key Words: Hydroxamic acids, biological activities, biochemical processes.

INTRODUCTION

Since their discovery by Wahlroos and Virtanen (1) in 1959, and over the past decades, the chemistry and biochemistry of hydroxamic acids and their derivatives have attracted considerable attention, due to their pharmacological, toxicological and pathological properties. Hydroxamic acids generally have low toxicities and have a wide spectrum of activities in all types of biological systems, as such they act variously as growth factors, food additives, tumor inhibitors, antimicrobial agents, antituberculous, antileukemic agents, key pharmacophore in many important chemotherapeutic agents, pigments and cell-division factors. Several of them have been advanced into human clinical trials as pharmaceutical drugs, for the treatment of several diseases. The following is a brief concerted attempt to describe the above roles of hydroxamate molecules in a variety of circumstances that are used widely in biology and medicine.

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INHIBITION EFFECT AND ANTICANCER ACTIVITY OF HYDROXAMIC ACIDS

The design and synthesis of ligands for biomedical applications in fields such as anticancer applications has become of great importance. One of these important ligands is hydroxamate molecules. Hydroxamic acids have been found to react with both proteins and nucleic acids (2). The reactivity of hydroxamic acids towards sulfhydryl groups of proteins has been suggested to be the reason for their inhibitory effect on various enzymes. The protease papain, for instance, with a single free cysteine residue located at the active site was irreversibly inhibited by DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one). Friebe and co-workers (3) showed an inhibitory effect of DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) and DIMBOA on plasma membrane H^+-ATPase from roots *A. sativa* and *Avena fatua*. This inhibition may also be due to the reactivity of hydroxamic acids towards sulfhydryl groups since at least one exposed cysteine residue at the active site is of importance for maintenance of enzyme conformation (3). In addition, DIMBOA was shown to have an inhibitory effect on the electron trans-

port and thus adenosine-tri-phosphate (ATP) production in isolated mitochondria and chloroplasts of maize (4). Both DIBOA and DIMBOA have been shown to be mutagenic in a test with *Salmonella typhimurium* (2). The reactivity of hydroxamic acids offers an explanation to the various biological effects observed.

The matrix metalloproteinases (MMP) are a family of zinc-dependent enzymes that are required for extra cellular matrix degradation and tissue remodeling. The ability of the hydroxamic acid functionality to form abidentate chelate with the zinc and nickel atoms in the enzyme's active site is considered to be an important functional feature metalloenzyme inhibition, namely as inhibitors of metalloproteinase (5), matric metalloproteinase (6, 7), they are also potent and specific inhibitors of urease activity (8, 9), thermolysin (10, 86), elastase (11), peroxidases (12), amino peptidases (13, 87).

It is well known from the literature (14–20, 88) that the unsubstituted aliphatic hydroxamic acids (such as acetohydroxamic acid) are well established as effective inhibitors of plant (14,15, 88), and bacterial urease *in vitro* (15,16) and have been shown to effectively inhibit ureolytic activity and/or to lower blood ammonia levels in mice (17), rats (15), sheep (18), cows (19), dogs and men (20). The potential application of these compounds in the treatment of hepatic coma and in the improvement of nitrogen utilization by ruminant animals has led to the present series of their physiologic disposition in the animal body. The four lower aliphatic hydroxamates were studied by chromatographic and spectrophotometric methods in unlabeled form (21).

Recently, significant advances have been made toward understanding the inhibition phenomena basis of hydroxamic acids. A number of hydroxamic acid analogues have been shown to inhibit DNA (dinucleic acid) synthesis by inactivating the enzyme ribonucleotide reductase (RNR) (22–25). This metalloenzyme catalyzes the conversion of (ribo) nucleotides to deoxy (ribo) nucleotides and is therefore a potential target for the development of anticancer agents (26–28). Hydroxamic acid moiety, R-CONHOH, is found to be the essential pharmacophore in the hydroxyurea, a clinically useful inhibitor of ribonucleotide reductase (29). A variety of nucleoside analogues are also active as inhibitors of ribonucleotide reductase (30, 32), following the inhibition mechanism similar to that proposed for hydroxamates (33-35). Farr *et al.* (37) designs and synthesizes a nucleoside analogue incorporating hydroxamate moiety. Their compounds inhibited RNR activity, but were 10-fold less potent than hydroxyurea. Moreover, recent reports (37–39) show that hydroxamate compounds increase the potency of nucleosides against HIV-1 (human-immunodeficiency-virus-1) give an additional importance to derivatives combining the structural features of the above compounds.

Quite recently, there have been articles concerning the biochemistry of anticancer activity of naturally occurring and synthetic class of organic compounds containing the hydroxamic acid functional group (-CONHOH). Hydroxyurea containing that group, is a well known anticancer drug (40–43). It inhibits the DNA synthesis by imparing the activity of enzyme ribonucleotide reductase (42–45). Though it is clinically used as anticancer agent, it perturbs the hematological parameters and depresses the bone marrow (46). Subsequently anticancer properties of some aliphatic and aromatic hydroxamic acids (such as acetohydroxamic acid, benzohydroxamic acid and salicylhydroxamic acid) have been studied (47, 48). Recently it has been reported that cholorohydroxamic acid possesses antitumor properties and inhibits the growth of Ehrlich ascites carcinoma (EAC) cells by imparing DNA and protein synthesis without altering the hematological parameters (49). No such studies have yet been done with acetohydroxamic acid, benzohydroxamic acid and salicylhydroxamic acid. The comparative study of antineoplastic activities and host toxic effects of hydroxamic acids (cholorohydroxamic acid, acetohydroxamic acid, benzohydroxamic acid, salicylhydroxamic acid and hydroxyurea) has been recently reported (50). In addition peritoneal macrophages and lipid peroxidation in normal mice after treatment with these hydroxamic acids have been presented (51). Transplantability of hydroxamic acid treated EAC cells has also been observed. The results show that cholorohydroxamic acid can be considered as the most effective antitumor agent amongst the hydroxamic acids studied and are comparable with hydroxyurea

regarding cell growth inhibition and survival time of tumor bearing mice. However, it is necessary that the antitumor activity of cholorohydroxamic acid should be carried out against different tumor cell lines which may bring promising results in cancer chemotherapy.

Recently a new oral chelator, salicylhydroxamic acid was developed and found to have promising advantages in the clinical treatment of thalassaemia major (52), as a trypanocidal drug (53) and used as inhibitors of viral growth (54), selective inhibition of deoxyribonucleic acid synthesis (55), also salicylhydroxamic acid inhibits delta 6 desaturation in the microalgo porphyridium cruentum (56) and also, the selective inhibition of catechol oxidases by salicylhydroxamic acid was reported (57). In addition to this, halogen substituted salicylhydroxamic acids used in lowering of rabbits (58).

In recent studies, it was reported that, benzohydroxamic acid had significant antitumor activity (59), substituted benzohydroxamic acid has been prepared to enhance the effect of benzohydroxamic acid (60, 90, 91) and its complexes with copper metal ions (Cu-benzohydroxamic acid) and used as a potential antitumor drug (61).

Because of the wide spread physiological importance of trihydroxamic acids, it is highly desirable to investigate several natural and synthetic trihydroxamic acids that will be selective for therapy of certain diseases. Desferrioxamine, a natural trihydroxamic acid, is a chelating of iron, aluminum and other metals, is used therapeutically for the treatment of iron-overloaded-patients (62, 92) to remove excess iron in patients suffering from iron overload as a consequence of the treatment of Cooley's anemia, or acute iron poisoning, particularly in patients with AIDS, but the lack of oral activity and its short biological half-life limits its use. Otherwise, iron chelation by desferrioxamine, and other chelators, protects against the cytotoxic and reactivating effects of hydrogen peroxide (63), and thus decreases NF-kB activation of HIV-1 transcription. Also, desferrioxamine B is used in human medicine removal of excess aluminum from the human body in those patients who must undergo permanent hemodialysis (64, 93).

More recently oxazole and oxadiazole hydroxamic acids are claimed by Pfizer as inhibitors of procollagen C-proteinase (PCP or pCP). Fundamental work on this enzyme has been carried out at Thomas Jefferson University, who described recombinant pCP and its use in treatment of fibrotic disorders. Until now, virtually the only indication that pCP inhibitors were targets for systematic drug discovery were from Roche, whose preferred candidates are also heterocyclyl hydroxamic acids, for example thiazoles.

Allelopathy, the chemical interaction of plants within the same species or between plants of different species, is important in the plant competition for water, nutrients and light. Hydroxamic acids have been ascribed a role in this interaction in many reports. DIBOA and BOA (2-benzoxazolinone) were shown to have an inhibitory effect on root growth of cress (*Lepidium sativum*) and barnyardn grass (*Echinochloa crusgalli*) (65). DIMBOA and MBOA (6-methoxy-2-benzoxazolinone) from *Triticum durum* were shown to have a growth inhibiting effect on roots of the weed *A. fatua* (66). In addition, MBOA was shown to inhibit seed germination on *A. fatua*. Rye root exudates containing DIBOA inhibited root growth of *A. fatua* whereas wheat root exudates without detectable amounts of hydroxamic acid did not (67).

A possible explanation to the growth inhibitory effect of hydroxamic acids on other plant species might be due to their ability to modify auxin action. Venis and Watson (68) reported that methylated benzoxazolinones are able to inhibit the binding of auxin to membrane receptors. MBOA was shown to have an inhibiting effect on auxin-induced bending (at concentrations of 0.6 mM or higher) and elongation (at concentrations of 0.6 mM) of oat coleoptiles (69). In contrast, DIMBOA had a supporting effect on auxin-induced elongation of maize coleoptiles at a concentration of 20 mM (70). The differences between the results of these experiments might be explained by different sensitivities of the plant species tested. Maize already contains DIMBOA and a UDP-glucose: Hx(hydroxamic acids)-glucosyltransferase (71, 72), to detoxify DIMBOA, that might interfere with the experiment. For instance, it has been shown that A. thaliana transformed with the genes for UDP-glucose: hydroxamic acid - glucosyltransferases isolated from maize are less sensitive to the allelopathic substances DIBOA and DIMBOA in growth assays than wt

A. thaliana that does not contain glucosyltransferases acting on these substances (72). Another possible explanation is that in maize only MBOA and not DIMBOA is a potent inactivator of auxin-induced shoot elongation (73). Maize bxbx mutant, deficient in DIMBOA synthesis grow normally, although extremely susceptible to pathogen attack (74). It has been shown, though, that the bxbx mutant still contains trace amounts of DIMBOA, which might be enough to maintain a normal growth (75). However DIMBOA, when exuded from Hx/HxGlc (hydroxamic acids/hydroxamic acid glucoside(s)) containing plants, may be more important in plant competition with other plants for water, light and nutrients by inhibiting the growth of neighboring plants than in growth regulation of the plant itself.

RESISTANCE ACTIVITY OF HYDROXAMIC ACIDS TOWARDS INSECTS

Hydroxamic acids have been shown to have a negative impact on the survival and reproduction of aphids. Argando–a et al. (76) found inverse correlations between hydroxamic acid content in different varieties of rye and wheat and the growth rate of the aphid *Metopolophium dirhodum*. When the plants grow older and the hydroxamic acid levels become decreased, the growth rate of the aphid populations are increased. In the same paper it was also reported that aphids fed with artificial diets containing DIMBOA or MBOA had lower survival rate than aphids fed on artificial diets lacking DIMBOA or MBOA. Correlations have also been shown in maize between high hydroxamic acid levels and resistance to the European corn borer *Ostrinia nubilalis* (77, 78). In addition to the reports based on correlations and work with insects on artificial diets, hydroxamic acids have been shown to be induced by infestation with insects. Hydroxamic acid levels in the wounded tissue of maize stems and leaves increased upon infestation with larvae of the corn borer Sesamia nonagrioides (79). In wheat, several cultivars including one *T. durum* cultivar showed increased levels of DIMBOA in leaf tissue upon infestation with the aphids *M. dirhodum* and *Rhopalosiphum padi* (2, 80). Induced changes in hydroxamic acids levels were however, later shown in wild wheat (*Triticum uniaristatum*) to be due to translocation rather than enhanced local synthesis of Hx (81).

ANTIFUNGAL ACTIVITY OF HYDROXAMIC ACIDS

As early as 1959, Wahlroos and Virtanen (1) reported about the anti-fungal effect of hydroxamic acids and their breakdown products on snow mold (Fusarium nivale). Fungi grown on medium containing DIMBOA, MBOA or BOA showed smaller colony diameter than fungi grown on an identical medium but lacking these compounds. Later reports indicated inverse correlations between infection ratings of Northern corn leaf blight-producing fungus (Helminthosporium turcicum) and plant DIMBOA levels, as well as inhibition of H. turcicum spore germination by DIMBOA (82, 83). These and other inhibitory effects of Hx on fungal growth are summarized by Niemeyer *et al.* (2). More recent studies of the fungal pathogen Gaeumannomyces graminis that causes the disease take-all in wheat and barley showed that DIBOA was a more potent fungal growth inhibitor than DIMBOA which is in correlation with the resistance of rye to take-all (84).

ANTIBACTERIAL ACTIVITY OF HYDROXAMIC ACIDS

Bacterial stalk rot of maize is caused by a certain strain of *Erwinia chrysanthemi*. Maize is, however, resistant to rot caused by other isolates of *Erwinia chrysanthemi* and other soft rotting *Erwinia* species. It has been shown that DIMBOA inhibits the growth of several soft rotting *Erwinia* species at concentrations of 0.2-0.3 mM and that strains non-pathogenic to maize were more sensitive to DIMBOA than pathogenic strains. DIMBOA was therefore proposed to be involved in the resistance towards *Erwinia* (85).

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REFERENCES

1. Wahlroos Ö, Virtanen AI : The precursors of 6-methoxybenzoxazolinone in maize and wheat plants, their isolation and some of their properties. Acta Chem Scand, 13, 1906, 1959.

2. Niemeyer HM, Pesel E, Copaja SV, Bravo HR, Franke S, Francke W : Changes in hydroxamic acid levels of wheat plants induced by aphid feeding. Phytochemistry, 28, 447, 1989.

3. Friebe A, Roth U, Kück P, Schnabl H, Schulz M : Effects of 2,4-dihydroxy-1,4-benzoxazin-3-ones on the activity of plasma membrane H^+-ATPase. Phytochemistry, 44, 979, 1997.

4. Massardo F, Zœ–iga GE, P rez LM, Corcuera LJ : Effects of hydroxamic acids on electron transport and their cellular location in corn. Phytochemistry, 35, 873, 1994.

5. Sawa M, Kiyoi T, Kurokawa K, Kumihara H, Yamamoto M, Miyasaka T, Ito Y, Hirayama R, Inoue T, Kirii Y, Nishiwaki E, Ohmoto H, Maeda Y, Ishibushi E, Inoue Y, Yoshino K, Kondo H : New type of metalloproteinase inhibitor: Design and synthesis of new phosphono hydroxamic acids. J Med Chem, 45, 4, 919, 2002.

6. Groneberg RD, Burns CJ, Morrissette MM, Ullrich JW, Morris RL, Darnbrough S, Djuric SW, Condon SM, McGeehan GM, Labaudiniere R, Neuenschwander K, Scotese AC, Kline JA : Dual inhibition of phosphodiesterase 4 and matrix metalloproteinases by an aryl hydroxamic acid template. J Med Chem, 42, 4, 541, 1999.

7. Aranapakam VJ, Davis M, Grosu GT, Baker JL, Ellingboe J, Zask A, Levin JI, Sandanayaka VP, Du M, Skotnicki JS, DiJoseph JF, Sung A, Sharr MA, Killar LM, Walter T, Jin G, Cowling R, Tillett J, Zhao W, McDevitt J, Xu ZB : Synthesis and structure activity relationship of N-substituted 4-arylsulfonylpipetidehydroxamic acids as novel, orally active matrix metalloproteinase inhibitors for treatment of Osteoarthritis. J Med Chem, 46, 12, 2376, 2003.

8. Parker MH, Lunney EA, Ortwine DF, Pavlovsky AG, Humblet C, Brouillette CG : Analysis of the binding of hydroxamic acid and carboxylic acid inhibitors to the stromelysin-1 (matrix metalloproteinase-3) catalytic domain by isothermal titration calorimetry. Biochemistry, 38, 41, 13592, 1999.

9. Toba S, Damodaran KV, Merz Jr KM : Binding Preferences of Hydroxamate Inhibitors of the Matrix Metalloproteinase Human Fibroblast Collagenase (HFC). J Med Chem, 42, 1225, 1999.

10. Nashino N, Powers JC : Phosphorus-Based SAHA Analogues as Histone Deacetylase Inhibitors. Biochemistry, 17, 2846, 1978.

11. Nashino N, Powers JC : Pseudomonas aeruginosa elastase. Development of a new substrate, inhibitors, and an affinity ligand. J Biol Chem, 255, 3482, 1980.

12. Tam SSC, Lee DHS, Wang EY, Munroe DG, Lau CY : Tepoxalin, a Novel Dual Inhibitor of the Prostaglandin-H Synthase Cyclooxygenase and Peroxidase Activities. J Biol Chem, 270, 13948, 1995.

13. Baker JO, Wilkes SH, Bayliss ME, Prescott JM : Hydroxamate-induced spectral perturbations of cobalt A Stereospecificity of amino acid hydroxamate inhibition of aminopeptidases eromonas aminopeptidase. Biochemistry, 22, 2098, 1983.

14. Kobashi K, Hase J, Uehera K : Specific inhibition of urease by hydroxamic acids. Biochem Biophysic Acta, 65, 380, 1962.

15. Fishbein WN, Carbone PP, Hochstein HD : Acetohydroxamate: Abcaterial urease inhibitors with therapeutic potential in hyperamonemic states. Nature, 208, 46, 1965.

16. Kobashi K, Hase J : Inhibition of proteus vulgaris urease by hydroxamic acids. J Biochem, 62, 293, 1967.

17. Fishbein WN : Urease inhibitor for hepatic coma: Inhibition of ^{14}C-urea hydrolysis in mice by alkylhydroxamate. I. Methodology. Biochem Med, 1, 111, 1967.

18. Streeter CL, Oltjen RR, Slyter LL, Fishbein WN : Urea utilization in weathers receiving the urease inhibitor, acetohydroxamic acid. J Anim Sci, 29, 88, 1969.

19. Brent BE, Adepoju A : Effect of acetohydroxamic acid on rumen ureases. J Anim Sci, 26, 1482, 1967.

20. Summerskill WN, Thorsell F, Feinberg J, Alderte JS : Effects of urease inhibition in hyperammonemia: Clinical and experimental studies with acetohydroxamic acid. Gastroenterology, 54, 20, 1968.

21. Fishbein WN, Streeter CL : Physiological disposition of short chain aliphatic hydroxamates in the mouse. J Pharmcol Exp Ther, 174, 239, 1970.

22. Valerije V, Vesna A, Stanko U : First Hydroxamic Seconucleoside Derivatives. Croat Chim Acta, 71, 119, 1998.

23. Larsen IK, Sjberg BM, Thelander L : Characterization of the active site of ribonucleotide reductase of Escherichia coli, bacteriophage T4 and mammalian cells by inhibition studies with hydroxyurea analogues. Eur J Biochem, 125, 75, 1982.

24. Riet B, Wampler GL, Elford HL : Synthesis of hydroxy- and amino-substituted benzohydroxamic acids: Inhibition of ribonucleotide reductase and antitumor activity. J Med Chem, 22, 589, 1979.

25. Elford HL, Wampler GL, Riet B : New ribonucleotide reductase inhibitors with antineoplastic-activity. Cancer Res, 39, 844, 1979.

26. Reichard P : Molecular Evaluation: From RNA to DNA, why so many ribonucleotide reductases? Science, 260, 1773, 1993.

27. Stubbe J : Ribonucleotide reductases. Adv Enzymol Relat Areas Mol Biol, 63, 349, 1990.

28. Stubbe J, Donk WA : Ribonucleotide reductases: Radical enzymes with suicidal tendencies. Chem Biol, 2, 793, 1995.

29. Donehower RC : Hydroxyurea. In: Cancer Chemotherapy. Principles and Practice, Ed by JM Chabner, JM Collins. JB Lippincott Co, Philadelphia, pp 225-233, 1990.

30. Gupta SP : Quantitative Structure-Activity Relationship Studies on Anticancer Drugs. Chem Rev, 94, 1507, 1994.

31. Xie KC, Plunkett W : Deoxynucleotide pool depletion and sustained inhibition of ribonucleotide reductase and DNA synthesis after treatment of human lymphoblastoid cells with 2-chloro-9-(2-deoxy-2-fluoro-b-D-arabinofuranosyl) adenine. Cancer Res, 56, 3030, 1996.

32. Lehmann TE, Berkessel A : Stereoselective Synthesis of 4'-Benzophenone-Substituted Nucleoside Analogs: Photoactive Models for Ribonucleotide Reductases. J Org Chem, 62, 302, 1997.

33. Salowe S, Bolliger JM, Ator M, Stubbe J, McCracken J, Peisach J, Samano MC, Robins MJ : An Alternative Model for Mechanism-Based Inhibition of E. Coli Ribonucleotide Reductase by 2' Azido-2' deoxyuridine 5'-diphosphate. Biochemistry, 32, 12749, 1993.

34. Robins MJ, Guo Z, Wnuk SF : J Am Chem Soc, 119, 3637, 1997.

35. Eliasson R, Pontis E, Eckstein F, Reichard P : J Biol Chem, 265, 26116, 1994.

36. Ingemarson R, Thelander L : A kinetic study on the influence of nucleoside triphosphate effectors on subunit interaction in mouse ribonucleotide reductase. Biochemistry, 35, 8603, 1996.

37. Farr RA, Bey P, Sunkara PS, Lippert BJ : The synthesis of acyclonucleoside hydroxamic acids as inhibitors of ribonucleotide reductase. J Med Chem, 32, 1879, 1989.

38. Malley SD, Grange JM, Hamedi SF, Vila JR : Identification and Characterization of Rat Intestinal Trefoil Factor: Tissue- and Cell-specific Member of the Trefoil Protein Family. Proc Natl Acad Sci, USA, 91, 11017, 1994.

39. Lori F, Malykh A, Cara A, Sun D, Weinstein JN, Lisziewicz J, Gallo RC : Subsequent research showed that in vitro anti-HIV activity is enhanced without increased toxicity when hydroxyurea is used with didanosine. Science, 266, 801, 1994.

40. Goa WY, Mitsuya H, Driscoll JS, Johns DG : Enhancement by hydroxyurea of the anti-human immunodeficiency virus type 1 potency of 2'-b-fluoro-2',3'-dideoxyadenosine in peripheral blood mononuclear cells. Biochem Pharmacol, 50, 274, 1995.

41. Gora TJ, Robak T : Clinical pharmacology of hydroxyurea. Acta Haematol Pol, 26, 39, 1995.

42. Coutinho LH, Brereton ML, Santos AM, Ryder WD, Chang J, Harrison CJ, Yin JA, Dekter TM, Testa NG : Evaluation of cytogenetic conversion to Ph-haemopoiesis in longterm bone marrow culture for patients with chronic myeloid leukemia on conventional hydroxyurea therapy, on pulse high-dose hydroxyurea and on interferonalpha. Br J Haematol, 93, 869, 1996.

43. Nand S, Stock W, Godwin J, Fisher SG : Leukemogenic risk of hydroxyurea therapy in polycythemia vera, essential thrombocythemia, myeloid metaplasia with myelofibrosis. Am J Haematol, 52, 42, 1996.

44. Santarossa S, Vaccher E, Lenerdon D, Marlo A, Errante D, Tirelli U : Ribonucleotide reductase inhibition in the treatment of adverse prostate cancer: An experimental approach with hydroxyurea and gallium nitrate in 20 patients. Eur J Cancer, 31A:959, 1995.

45. Krakoff IH, Brown NC, Reichard P : Inhibition of ribonucleotide diphosphatase reductase by hydroxyurea. Cancer Res, 28, 1959, 1986.

46. Elford H : Effect of hydroxyurea on ribonucleotide reductase. Biochem Biophys Res Commun, 33, 129, 1968.

47. Bruce P, Kennedy BJ : Duration of DNA inhibition by hydroxyurea. Proc Am Asso Cancer Res, 11, 63, 1970.

48. Moore EC : The effects of ferrous ion and dithioerythritol on inhibition by hydroxyurea of ribonucleotide reductase. Cancer Res, 29:291, 1969.

49. Elford HL, Wampler GL, Riet BV : New ribonucleotide reductase inhibitors with antineoplastic-activity. Cancer Res, 39, 844, 1971.

50. Sur P, Bag SP, Sur B, Khanam JA : Chloroacetohydroxamic acid as antitumor agent against Ehrlich ascites carcinoma in mice. Neoplasma, 44, 197, 1997.

51. Khanam JA, Bag SP, Sur B, Sur P : Comparative study of antineoplastic activity of some aliphatic and aromatic hydroxamic acids against ahrlich ascites carcinoma (EAC) in mice. Med J Islamic Acad Sciences, 11, 2, 1998.

52. K Quirolo : www.thassalemia.com/transfusion/chelation.html. Children's Hospital of Oakland.

53. Opperdoes FR, Aarsen PN, Meer CV, Borst P : Trypanosoma brucei: An evaluation of salicylhydroxamic acid as a trypanocidal drug. Exp Parasital, 40, 2, 198, 1976.

54. Scheel M, Pfeffer R : Inhibition of viral growth by salicylhydroxamic acid. Proc Soc Exp Biol Med, 128, 3, 902, 1968.55. Gale GR : Selective inhibition of deoxyribonucleic acid synthesis by salicylhydroxamic. Proc Exp Biol Med, 122, 4, 1236, 1966.

56. Khozin-Goldberg I, Bigogno C, Cohen Z : Salicylhydroxamic acid inhibits delta6 desaturation in the microalga Porphyridium cruentum. Biochim Biophys Acta, 1439, 384, 1999.

57. Andrew CA, John LW : The selective inhibition of catechol

oxidases by salicylhydroxamic acid. Phytochemistry, 27, 10, 3075, 1988.

58. Czyzyk A, Ostaszynski A, Plenkiewicz ZS, Urbanski T : Lowering of cholesterol level in the blood serum of rabbits by halogen substituted salicylhydroxamic acids. Arzneimittelforschung, 22, 2, 465, 1972.

59. Elford HL, Wampler GL, Riet BV : New ribonucleotide reductase inhibitors with antineoplastic activity. Cancer Res, 39, 844, 1979.

60. Riet BV : Synthesis of hydroxy and amino-substituted benzohydroxamic acids: Inhibition of ribonucleotide reductase and antitumour activity. J Med Chem, 22, 589, 1979.

61. Khanam JA, Bug SP, Sur B, Sur P : Antineoplastic activity of copper(II)-benzohydroxamic acid complex against Ehrlich Ascites Carcinoma (EAC) in mice. Indian J of Pharmacology, 29, 157, 1997.

62. Anne FV, Andrew JW, Marvin JM : Iron chelators from mycobacteria (1954-1999) and potential therapeutic applications. Nat Prod Rep, 17, 99, 2000.

63. Sappey C, Boelaert JR, Legrand-Poels S, Forceille C, Favier A, Piette J : AIDS Res Hum Retroviruses, 11, 1049, 1995.

64. Anderson WF, Bank A, Zaino EC (Ed) : Fourth Coolaeys Anemia Symposium. Ann N Y Acad Sci, 344, 448, 1980.

65. Klun JA, Guthrie WD, Hallauer AR, Russell WA : Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy 2H-1,4-benzoxazin-3 (4H)-one and resistance to the European corn borer in a diallel set of eleven maize inbreads. Crop Sci, 10, 87, 1970.

66. Gutierrez C, Castañera P, Torres V : Wound-induced changes in DIMBOA (2,4 dihydroxy-7-methoxy-2H-1,4 benzoxazin-3(4H)-one) concentration in maize plants caused by Sesamia nonagrioides (Lepidoptera: Noctuidae). Ann Appl Biol, 113, 447, 1988.

67. Gianoli E, Niemeyer HM : Characteristics of hydroxamic acid induction in wheat triggered by aphid infestation. J Chem Ecol, 23, 2695, 1997.

68. Venis MA, Watson PJ : Naturally occurring modifiers of auxin-receptor interaction in corn: Identification as benzoxazolinones. Planta, 142, 103, 1978.

69. Hasegawa K, Togo S, Urashima M, Mizutani J, Kosemura S, Yamamura S : An auxine-inhibiting substance from light-grown maize shoots. Phytochemistry, 31, 3673, 1992.

70. Park WJ, Schäfer A, Prinsen E, Onckelen H, Kang BG, Hertel R : Auxin-induced elongation of short maize coleoptile segments is supported by 2,4-dihydroxy-7-methoxy-1,4 benzoxazin-3-one. Planta, 213, 92, 2001.

71. Bailey BA, Larson RL : Hydroxamic acid glucosyltransferases from maize seedlings. Plant Physiol, 90, 1071, 1989.

72. Rad U, HŸttl R, Lottspeich F, Gierl A, Frey M : Two glucosyltransferases are involved in detoxification of benzoxazinoids in maize. Plant, J 28, 633, 2001. 73. Hoshi-Sakoda M, Usui K, Ishizuka K, Kosemura S, Yamamura S, Hasegawa K : Structure-activity relationships of benzoxazolinones with respect to auxin-induced growth and auxin-binding protein. Phytochemistry, 37, 297, 1994.

74. Frey M, Chomet P, Glawischnig E, Stettner C, Grün S, Winklmair A, Eisenreich W, Bacher A, Meeley RB, Briggs SP, Simcox K, Gierl A : Analysis of a chemical plant defense mechanism in grasses. Science, 277, 696, 1997.

75. Melanson D, Chilton MD, Masters-Moore D, Chilton WS : A deletion in an indole synthase gene is responsible for the DIMBOA-deficient phenotype of bxbx maize. Proc Natl Acad Sci, USA, 94, 13345, 1997.

76. Argandoña VH, Luza JG, Niemeyer HM, Corcuera LJ : Role of hydroxamic acids in the resistance of cereals to aphids. Phytochemistry, 19, 1665, 1980.

77. Klun JA, Robinson JF : Concentration of two 1,4-benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. J Econ Entomol, 62, 214, 1969.

78. Klun JA, Guthrie WD, Hallauer AR, Russell WA : Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy 2H-1,4-benzoxazin-3 (4H)-one and resistance to the European corn borer in a diallel set of eleven maize inbreads. Crop Sci, 10, 87, 1970.

79. Gutierrez C, Castañera P, Torres V : Wound-induced changes in DIMBOA (2,4 dihydroxy-7-methoxy-2H-1,4 benzoxazin-3(4H)-one) concentration in maize plants caused by Sesamia nonagrioides (Lepidoptera: Noctuidae). Ann Appl Biol, 113, 447, 1988.

80. Gianoli E, Niemeyer HM : Characteristics of hydroxamic acid induction in wheat triggered by aphid infestation. J Chem Ecol, 23, 2695, 1997.

81. Gianoli E, Niemeyer HM : Lack of costs of herbivory-induced defenses in a wild wheat: integration of physiological and ecological approaches. OIKOS, 80, 269, 1997.

82. Couture RM, Routley DG, Dunn GM : Role of cyclic hydroxamic acids in monogenic resistance of maize to Helminthosporium turcicum. Physiol Plant Path, 1, 515, 1971.

83. Long BJ, Dunn GM, Routley DG : Relationship of hydroxamic acid content in maize and resistance to Northern corn leaf blight. Crop Sci, 15, 333, 1975.

84. Wilkes MA, Marshall DR, Copeland L : Hydroxamic acids in cereal roots inhibit the growth of take-all. Soil Biol Biochem, 31, 1831, 1999.

85. Corcuera LJ, Woodward MD, Helgeson JP, Kelman A : 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one, an inhibitor from Zea mays with differential activity against soft rotting Erwinia species. Plant Physiol, 61, 791, 1978.

86. Rasnick D, Powers JC : Active site directed irreversible inhibition of thermolysin. Biochemistry, 17, 4363, 1978.

87. *Wilkes SH, Prescott JM : Stereospecificity of amino acid hydroxamate inhibition of aminopeptidases. J Biol Chem, 258, 13517, 1983.*

88. Fishbein WN, Daly JE : Urease inhibitor for hepatic coma: Comparative efficacy of four lower hydroxamate homologes in vitro and in vivo. Proc Soc Exp Biol Med, 134, 1083, 1970.

89. Natelson S, Pantazis P, Natelson EA : L-homoserine hydroxamic acid as an antitumor agent. Clin Chim Acta, 229, 133, 1994.

90. Tihon H, Elford L, Cory JC : Studies on the mechanisms of inhibition of L1210 cell growth by 3,4-dihydroxybenzohydroxamic acid and 3,4-dihydroxybenzamidoxim. Adv Enzyme Regul, 31, 71, 1991.

91. Hall HI, Izydore R, Hall ES, Miller MC, Daniels DL, Debnath ML, Woodard T : The antineoplastic and cytotoxicity of benzohydroxamic acid and related derivatives in murine and human tumour cells. Anti Cancer Drugs, 3, 273, 1992.

92. Guido CV, Roberta S, Gavino F : Oral iron chelators for clinical use. Polyhedron, 18, 25, 3219, 1999.

93. Crapper Mclachlan DR, Farnell B, Gallin H, Karlik S, Eichorn G, De Boni U : In Biological Aspects of Metals and Metal-Related Diseases, Ed by B Sarker, Raven Press, New York, 1983.

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