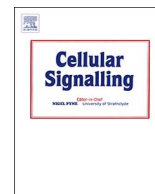




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Review

Niclosamide: Beyond an antihelminthic drug

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ABSTRACT

Niclosamide is an oral antihelminthic drug used to treat parasitic infections in millions of people worldwide. However recent studies have indicated that niclosamide may have broad clinical applications for the treatment of diseases other than those caused by parasites. These diseases and symptoms may include cancer, bacterial and viral infection, metabolic diseases such as Type II diabetes, NASH and NAFLD, artery constriction, endometriosis, neuropathic pain, rheumatoid arthritis, sclerodermatous graft-versus-host disease, and systemic sclerosis. Among the underlying mechanisms associated with the drug actions of niclosamide are uncoupling of oxidative phosphorylation, and modulation of Wnt/ β -catenin, mTORC1, STAT3, NF- κ B and Notch signaling pathways. Here we provide a brief overview of the biological activities of niclosamide, its potential clinical applications, and its challenges for use as a new therapy for systemic diseases.

1. Discovery of niclosamide as an anthelmintic drug

Niclosamide was discovered in the Bayer chemotherapy research laboratories in 1953. It was originally developed as a molluscicide to kill snails, an intermediate host of schistosomiasis, and was marketed as Bayluscide in 1959 [1]. In 1960, scientists at Bayer found it to be effective against human tapeworm (cestoda) infection, and it was marketed as Yomesan for human use in 1962 [1,2]. Niclosamide was approved by the US FDA for use in humans to treat tapeworm infection in 1982 and is included in the World Health Organization's list of essential medicines [3]. It has been used to safely treat millions of patients. For such a widely-used drug, Niclosamide's mechanism of action has not been well-delineated, although it has been reported to involve uncoupling of oxidative phosphorylation [4–7]. In the past several years, mounting evidence has accumulated that niclosamide is a multifunctional drug that is able to inhibit or regulate multiple signaling pathways and biological processes, suggesting that it may be developed as a novel treatment for more than just helminthic disease.

2. Niclosamide and cancer

2.1. Adrenocortical carcinoma

Adrenocortical carcinoma is a rare aggressive endocrine cancer and

surgical resection is the only effective therapy but has limited benefits [8]. Satoh et al. screened a small molecule library that contained 2492 drugs approved for human use with a luciferase-coupled ATP quantitation assay to assess cell viability [9]. Niclosamide was found to inhibit adrenocortical carcinoma cellular proliferation, which was associated with apoptosis, reduction of epithelial-to-mesenchymal transition and β -catenin levels. In addition, mitochondrial uncoupling activity was observed in cancer cells. Oral administration of niclosamide led to tumor growth inhibition with no observed toxicity.

2.2. Niclosamide and breast cancer

Breast cancer is a leading cause of death in women [10]. Development of new therapies will be necessary to reduce mortality. Lu et al. reported that niclosamide inhibits Wnt/ β -catenin signaling by promoting Wnt co-receptor LRP6 degradation in breast cancer cells [11]. Subsequently this group reported that niclosamide acts synergistically with a monoclonal antibody that specifically activates TRAIL death receptor 5 to inhibit tumor growth of basal-like breast cancers [12]. Fonseca et al. reported that Niclosamide inhibits mTORC1 signaling in MCF-7 breast cancer cells. Mechanistic studies indicated Niclosamide lowered the cytoplasmic pH and may indirectly lead to inhibition of mTORC1 signaling [13]. Niclosamide also was found to prevent the conversion of non-breast cancer stem cells into cancer stem cells [14]. This mechanism is associated with inhibition of the IL6-JAK1-STAT3

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signal transduction pathway. Ren et al. identified niclosamide as a potent STAT3 inhibitor able to suppress STAT3 transcriptional activity, using a cell-based STAT3-dependent dual luciferase reporter assay [15]. Wang et al. used a high-throughput drug screen of breast cancer spheroid growth and found that niclosamide inhibited the formation of breast cancer spheroids and induced apoptosis in vitro and tumor growth in vivo [16]. Karakas et al. reported that niclosamide enhanced the antitumor activity of a palladium (II) saccharinate complex of terpyridine, leading to enhanced cytotoxic activity in breast cancer stem cells [17].

Triple-negative breast cancer is defined by the lack of expression of the estrogen and progesterone receptors and the lack of HER2 amplification. It accounts for about 15% of breast cancers and lacks effective therapies [18]. Yin et al. reported that niclosamide inhibits ionizing radiation-induced Wnt/ β -catenin signaling in triple-negative breast cancer cells in vitro and in vivo [19]. Liu et al. reported that niclosamide alone or in combination with cisplatin represses the growth of xenografts of cisplatin-resistant triple-negative breast cancer cells. They found that niclosamide reversed the epithelial-to-mesenchymal transition phenotype, inhibited Akt, ERK, and Src signaling pathways, and inhibited the proliferation of both cisplatin-sensitive (CS) and cisplatin-resistant (CR) triple-negative breast cancer 231 cells in vitro. Niclosamide alone or in combination with cisplatin also could repress the growth of xenografts in mice bearing either 231-CS or 231-CR cells [20].

2.3. Niclosamide and colon cancer

Colorectal cancer is the second leading cause of cancer-related deaths in the United States [21]. Current chemotherapy regimens do not target one of the most important underlying pathological mechanisms: the Wnt signaling pathway [22]. No FDA approved drug targets this pathway, which functions by Wnt ligands binding to cell surface Frizzled receptors to activate disheveled proteins that stabilize β -catenin from constitutive degradation by the APC complex. This allows β -catenin to accumulate and translocate to the nucleus to alter gene transcription through Tcf/Lef transcription factors. We screened a small molecule library containing 1200 FDA-approved drugs in a high throughput screen using Frizzled1-GFP internalization from the cell surface, and identified niclosamide as a small molecule inhibitor of Wnt/ β -catenin signaling [23]. Niclosamide promoted Wnt receptor Frizzled1 endocytosis, downregulated the Disheveled2 protein, inhibited Wnt3A-stimulated cytosolic β -catenin stabilization and inhibited Tcf/Lef gene reporter activity. Niclosamide inhibited growth of colon cancer cells from human patients both in vitro and in vivo, regardless of mutations in APC [24].

S100A4 is a known Wnt responsive gene. Sack et al. created an S100A4 promoter-driven luciferase reporter assay in human colorectal cancer HCT116 cells in order to screen a chemical library for compounds affecting S100A4 gene transcription. They identified niclosamide as an inhibitor of S100A4 gene transcription. Niclosamide reduced S100A4 mRNA and protein expression, and inhibited colon cell migration, invasion, proliferation, and colony formation in vitro, and also reduced liver metastasis in a mouse model [25]. Suliman et al. measured growth inhibition and apoptosis of three colon cancer cell lines (HCT116, Lovo, ad SW620) after treatment with niclosamide. They found that niclosamide treatment was associated with inhibition of the Notch signaling pathway and with increased expression of the tumor suppressor miR-200 family [26].

2.4. Niclosamide and glioma

Glioblastoma is the most common primary brain tumor and is responsible for the highest mortality among brain tumors [27]. Current therapies are not effective in reducing mortality [28]. Wieland et al. employed a cell viability assay to screen a small collection of 160

synthetic and natural compounds, and found niclosamide selectively inhibited glioblastoma cell viability [29]. Detailed mechanism studies revealed that niclosamide suppressed the Wnt, Notch, mTOR, and NF- κ B signaling pathways. Pre-exposure to niclosamide significantly diminished the malignant potential of glioma cells in vivo.

2.5. Niclosamide and head and neck cancer

Head and neck cancers are a group of biologically similar cancers arising in the lip, oral cavity, nasal cavity, pharynx, larynx and paranasal sinuses. The most common type of head and neck cancer is squamous cell carcinoma. Head and neck cancer accounts for about 3% of all cancer deaths in the United States [30]. Li et al. reported that inhibition of EGFR by erlotinib, an FDA-approved therapeutic agent, led to activation of STAT3 signaling in head and neck cancer cells, an effect that may be responsible for reduced therapeutic efficacy of erlotinib against head and neck cancers. The ability of niclosamide to inhibit STAT3 signaling led to growth inhibition of head and neck cancer cells in vitro and in vivo, and an enhanced anti-tumor effect of erlotinib [31].

2.6. Niclosamide and leukemia

Leukemias are cancers of bone marrow stem cells or bone marrow-derived progenitors that result in high numbers of abnormal white blood cells in circulation and in blood-forming tissues, including the bone marrow and the lymphatic system. About 50,000 cases of leukemia are diagnosed annually in the U.S. [30].

The Notch signaling pathway is important in the generation of hematopoietic stem cells. Activated Notch receptors are cleaved to release the Notch intracellular domain (NICD) which moves to the nucleus and binds to transcription factors such as CBF1 to alter gene expression [32]. Wang et al. employed a CBF1-driven luciferase reporter system to search for small molecule modulators of CBF1-dependent Notch signaling. They identified niclosamide as an inhibitor of endogenous Notch signaling in AML cells, a cell line derived from an acute myelogenous leukemia patient [33]. Jin et al. determined that niclosamide inhibits TNF- α -induced NF- κ B-dependent reporter activity and increased the levels of reactive oxygen species (ROS) in AML cells. Niclosamide was synergistic with chemotherapeutic agents cytarabine, etoposide, and daunorubicin in vitro, and inhibited the growth of AML cells implanted in nude mice [34]. Recently Jin et al. reported that niclosamide decreased the long-term engraftment of chronic myelogenous leukemia (CML) CD34⁺ stem cells implanted in immunodeficient NOG mice, and prolongs the survival of mice bearing leukemia cells driven by the human Bcr-Abl gene fusion, a common chromosomal translocation mutant driver of leukemia. The mechanism may involve the disruption of a positive feedback loop between NF- κ B and FOXM1/ β -catenin resulting in impaired self-renewal capacity and survival of CML [35].

2.7. Niclosamide and lung cancer

Lung cancer is the second most common cancer, but has the highest mortality rate in the United States [30]. About 20% of patients with non-small cell lung cancer (NSCLC) harbor mutations in the EGFR gene, which promotes cancer cell growth. EGFR inhibitors (e.g. erlotinib) have been deployed, but drug resistance has emerged to mitigate the effectiveness of this class of agents [36]. Li et al. found that erlotinib resistance was associated with activation of STAT3-Bcl2-Bcl-XL signaling. Niclosamide treatment overcame erlotinib resistance. Niclosamide in combination with erlotinib potently repressed growth of erlotinib-resistant lung cancer cell xenografts and increased apoptosis in tumors [37]. The same research group subsequently reported that niclosamide is effective in reducing radio-resistance of human lung cancers in vitro and in vivo. The mechanism involves inhibition of JAK2-STAT3 activity that is induced by radiation [38]. Lee et al. used a

cell viability screen to determine that niclosamide enhanced radiosensitivity of the non-small cell lung cancer cell line H1299. This suggests that niclosamide may be useful as a radiosensitizer in lung cancer patients [39].

2.8. Niclosamide and osteosarcoma

Osteosarcoma is the most common primary bone malignancy [40]. Osteosarcoma patient survival has improved minimally despite advances in surgical techniques and chemotherapeutic regimens. Liao et al. reported that niclosamide can effectively inhibit osteosarcoma cell proliferation, migration, and survival. This inhibitory effect is associated with decreased expression of c-Fos, c-Jun, E2F1, and c-Myc. Niclosamide also inhibits osteosarcoma tumor growth in a mouse xenograft tumor model [41].

2.9. Niclosamide and ovarian cancer

Ovarian cancer accounts for 3% of all cancers in women [30]. Yo et al. screened a bioactive compound library for inhibition of spheroid formation by cisplatin-resistant CP70 ovarian cancer cells, and identified niclosamide as an active drug in this assay. Subsequently, niclosamide was found to inhibit ovarian tumor-initiating cells in vitro and in vivo through alteration of metabolic pathways [42].

Haygood et al. isolated ovarian cancer tumor spheres from patients and treated them concurrently with niclosamide (or analogs) and carboplatin, and observed cytotoxicity by this combination. In the drug-treated samples, Wnt responsive genes were inhibited [43,44]. King et al. showed that Wnt7A and FGF1 expression are highly correlated in ovarian carcinomas, and FGF1 is a direct transcriptional target of Wnt7A/ β -catenin signaling. Niclosamide abrogated Wnt7A/ β -catenin signaling, decreased β -catenin transcriptional activity and cell viability and increased cell death. Oral niclosamide inhibited tumor growth and the progression of human ovarian cancers in xenograft animal models [45].

2.10. Niclosamide and prostate cancer

Prostate cancer is the most common cancer among men in the United States, and is a leading cause of cancer death among men [30]. Many prostate tumors are androgen-dependent, so anti-androgens are important therapeutics [46]. Enzalutamide is a new anti-androgen for the treatment of metastatic, castration-resistant prostate cancer [47]. Resistance to enzalutamide therapy was reported to be associated with the expression of androgen receptor splice variants, including the AR-V7 isoform [48]. Liu et al. employed an androgen-stimulated luciferase activity assay to identify potential drugs against the activity of the AR-V7 isoform [49]. They found that niclosamide downregulated AR-V7 protein expression and inhibited AR-V7 transcription activity, and reduced the recruitment of AR-V7 to the prostate-specific antigen promoter. Niclosamide also inhibited prostate cancer cell growth in vitro and tumor growth in vivo, and synergized with enzalutamide to inhibit growth of enzalutamide-resistant tumors, indicating its potential application for patients with enzalutamide resistance. Subsequently Liu et al. found that niclosamide inhibited STAT3 activation in prostate cancer cells [50]. Niclosamide synergistically reversed enzalutamide resistance in prostate cancer cells and combination treatment with niclosamide plus enzalutamide resulted in inhibition of colony formation and growth arrest by inducing cell apoptosis. The mechanism by which niclosamide overcomes enzalutamide resistance may be associated with down-regulation of STAT3 target genes and preventing recruitment of the androgen receptor to the prostate-specific antigen promoter in prostate cancer cells in an IL6-dependent manner. These results suggest that niclosamide may target the IL6-STAT3-AR pathway to overcome enzalutamide resistance to inhibit tumor cell migration and invasion in advanced prostate cancer [50].

It is known that therapeutic agents (including androgen-deprivation therapy), chemotherapeutic agents, and radiotherapy induce neuroendocrine differentiation in prostate cancer cells. These differentiated cells lack expression of the androgen receptor and prostate specific antigen, and are resistant to treatments [51]. Ippolito et al. reported that the ability of niclosamide to inhibit mitochondrial function is associated with acidic pH in prostate neuroendocrine cancer cells. Niclosamide has pH-dependent toxicity in a castration-resistant neuroendocrine prostate cancer cell line [52].

2.11. Niclosamide and renal cell carcinoma

Renal cell carcinoma originates from the epithelium of renal tubules. It is the most common type of kidney cancer in adults, and is resistant to currently available therapies [53,54]. Zhao et al. discovered that niclosamide inhibits proliferation and anchorage-independent colony formation in two renal cell carcinoma cell lines. Niclosamide synergized with cisplatin to reduce tumor growth in two in vivo renal cell carcinoma xenograft mouse models through a mechanism that involved both decreased β -catenin expression and mitochondrial dysfunction [55].

2.12. Summary of Niclosamide's mechanisms of action in cancer

The number of studies investigating the use of niclosamide as a potential therapeutic agent in various cancer tumor types is growing rapidly. A summary of niclosamide's mechanisms of action in cancer cited within is provided in Table 1.

3. Niclosamide and bacteria

3.1. Niclosamide and tuberculosis

Tuberculosis is the second-most common cause of death from infectious disease [56]. In an effort to overcome multidrug resistance

Table 1
Summary of Niclosamide's mechanisms of action in cancer.

Cancer	Mechanism of action	Reference
Adrenocortical carcinoma	β -catenin downregulation	[9]
Breast cancer	Mitochondrial uncoupling Wnt co-receptor LRP6 degradation	[11–16]
Colon cancer	IL-6-JAK1-STAT3 pathway inhibition Akt, ERK, and Src pathway inhibition Inhibition of mTORC1 signaling Frizzled1, Dvl2, Wnt/ β -catenin pathway inhibition	[23–26]
Glioma	S100A4 expression inhibition Notch signaling inhibition	[29]
Head and neck	Inhibition of Wnt, Notch, mTOR, and NF- κ B signaling	[31]
Leukemia	Inhibition of STAT3 signaling Inhibition of Notch signaling and NF- κ B pathway	[33–35]
Lung cancer	Reactive oxygen species (ROS) induction FOXM1/ β -catenin down regulation Inhibition of STAT3/Bcl2/Bcl-XL signaling pathway	[37–39]
Osteosarcoma	ROS and c-Jun activation in combination with ionizing radiation	[41]
Ovarian cancer	Inhibition of the expression of c-Fos, c-Jun, E2F1, and c-Myc	[42–45]
Prostate cancer	Inhibition of Wnt/ β -catenin Alteration of metabolic pathways	[50–52]
Renal cell carcinoma	Downregulation of AR-V7 protein expression IL6-Stat3-AR pathway inhibition Intracellular acidification induction Inhibition of Wnt/ β -catenin signaling	[55]

to current therapies, Sun et al. tested the ability of antifungal and antihelminthic drugs to inhibit the growth of *M. tuberculosis* strain H37Ra. Niclosamide was found to inhibit growth with a minimum inhibitory concentration of 0.5–1 μM . The authors suggested its topic use to treat surface-located tuberculosis, i.e. skin or intestinal tuberculosis infections [57]. Subsequently, a number of research groups have reported the ability of niclosamide and related salicylanilide derivatives to inhibit the growth of *M. tuberculosis* and reported the effect of pH on growth inhibition [58–60].

3.2. Niclosamide and anthrax

Anthrax is a zoonotic disease caused by infection by *Bacillus anthracis*. Despite the development of an anthrax vaccine, the disease remains a public health threat [61]. Zhu et al. used an established image-based assay that monitored the endocytosis and translocation of a beta-lactamase-fused anthrax lethal factor to identify small molecules that blocked anthrax toxin internalization [62]. They found that niclosamide protected RAW264.7 macrophages and CHO cells exposed to anthrax lethal toxin, and also defended cells from *Pseudomonas* exotoxin and diphtheria toxin. One of the mechanisms of niclosamide action may involve endosome acidification [13,62].

3.3. Niclosamide and *Pseudomonas aeruginosa*

Many bacteria use quorum sensing to coordinate certain behaviors such as biofilm formation, virulence, and antibiotic resistance [63]. Imperi et al. screened a library of FDA-approved drugs for their ability to inhibit the quorum sensing response in the Gram-negative pathogen *Pseudomonas aeruginosa*. They identified niclosamide as an inhibitor of the *P. aeruginosa* quorum sensing response, and of production of acyl-homoserine lactone, a quorum sensing signaling molecule. Niclosamide affected the transcription of about 250 genes in *P. aeruginosa* with a high degree of target specificity toward quorum sensing-dependent genes. It also suppressed surface motility and production of the secreted virulence factors elastase, pyocyanin, and rhamnolipids, and it reduced biofilm formation. Niclosamide also protected *Galleria mellonella* moth larvae from *P. aeruginosa* infections [64].

3.4. Niclosamide and *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive bacterium, and methicillin-resistant *S. aureus* (MRSA) is mainly responsible for hospital and community-acquired infections [65]. Rajamuthiah et al. established a *Caenorhabditis elegans* whole animal liquid MRSA infection high throughput screening assay to identify small molecules that prolong survival of infected *C. elegans* nematodes [66]. They screened the Biomol 4 library of 640 FDA-approved drugs, and niclosamide was one of the positive hits that prolonged nematode survival. Niclosamide inhibited the growth of methicillin-resistant *S. aureus*, as well as another Gram-positive bacteria *Enterococcus faecium*, but did not have any effect against the Gram-negative species *Pseudomonas aeruginosa*. Niclosamide was shown to be bacteriostatic. Oxyclozanide, a related salicylanilide derivative, was also effective against MRSA bacteria and was shown to be bactericidal. Thus, niclosamide may have utility in treating methicillin-resistant *S. aureus* (MRSA) infection.

4. Niclosamide and viral infections

Pandemic viral infections are an important public health threat. Strategies for controlling viral infections mainly use two approaches: agents that target the virus directly or agents that target the host [67]. Niclosamide has been reported as a potential agent for host defense during viral infections. Wu et al. screened a small chemical library consisting of marketed drugs for their ability to prevent infection by the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV). They

found that niclosamide inhibited SARS-CoV replication and protected Vero E6 cells from cytopathic effects after virus infection [68]. Niclosamide's effect on anti-viral host defense mechanisms was first reported by Jurgeit et al. They used a monoclonal antibody mabJ2 to stain viral dsRNA in infected cells as a readout for imaged-based screening [69]. They screened a library of 1200 known bioactive compounds and identified niclosamide as a potent, low micromolar inhibitor of pH-dependent human rhinoviruses (HRV) and influenza virus [70]. The mechanism of action proposed was related to niclosamide's protonophore activity and its ability to act as a proton carrier [70] as previously described [13]. Niclosamide thus could be a candidate for host-directed antiviral therapies.

Chikungunya virus is a member of the family *Togaviridae* and enters cells through receptor-mediated endocytosis [71]. Wang et al. used a Chikungunya virus 26S-mediated insect cell fusion inhibition assay as a high-throughput assay to screen a FDA-approved drug library, and identified niclosamide as having anti-Chikungunya virus activity through reducing Chikungunya virus entry and transmission [72].

Zika virus (ZIKV), a mosquito-borne flavivirus, is a growing public health concern following a large outbreak that started in Brazil in 2014 [73]. Xu et al. used ZIKV-induced caspase-3 activity in SNB-19 cells as a drug screen for inhibitors, and identified niclosamide as an inhibitor of ZIKV replication in 3D brain organoids. Combination treatment of niclosamide plus PF-03491390, a non-selective (pan-caspase) inhibitor of caspase activity, further increased protection of human neural progenitors and astrocytes from ZIKV-induced cell death [74].

5. Niclosamide and metabolic syndrome

Metabolic syndrome is a series of metabolic abnormalities associated with surplus energy intake, obesity, and sedentary lifestyle, and is a growing public health threat and clinical challenge worldwide. Patients with metabolic syndrome have higher risk of Type 2 diabetes mellitus and are predisposed to nonalcoholic fatty liver disease [75,76].

5.1. Niclosamide and Type 2 diabetes mellitus

Type 2 diabetes mellitus affects more than 25 million Americans, and is the seventh leading cause of death in the U.S. [77]. While lifestyle changes can have an impact in managing diabetes and medications can have effective outcomes, some patients often become refractory to therapy. As an antihelminthic drug, niclosamide was reported to be an uncoupler of oxidative phosphorylation [4,5] and to disrupt the pH homeostasis of the parasite to kill worms [78]. To seek new avenues for diabetes treatment, Tao et al. first demonstrated that a more water soluble form of niclosamide, niclosamide ethanolamine salt, uncouples mammalian mitochondria [79]. They added niclosamide ethanolamine salt to the food of mice fed a high fat diet in order to achieve drug exposure in vivo and overcome niclosamide's low exposure in mice when dosed intermittently [24]. They found that niclosamide ethanolamine salt treatment led to reduction in metabolic symptoms, an increased rate of energy expenditure, elevated oxygen consumption rate, and increased lipid oxidation. Niclosamide ethanolamine salt prevented elevation of fasting blood glucose and basal plasma insulin concentrations while improving insulin sensitivity and reducing body weight gain in mice fed with a high fat diet. In the established high fat diet diabetes mouse model, niclosamide ethanolamine salt treatment reversed metabolically deleterious effects. Similar results were observed in db/db diabetic mice in which diabetes develops due to a mutation in the leptin receptor gene.

5.2. Niclosamide and nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease is an early indication of the metabolic syndrome where lipids abnormally accumulate in the liver [76]. About 15–30% of the world's population is affected by nonalco-

holic fatty liver disease, a leading cause for Type 2 diabetes, nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma [80]. In addition to effects on diabetic symptoms, Tao et al. also described the effect of niclosamide ethanolamine salt to reduce liver fat accumulation (steatosis) in mice fed a high fat diet plus niclosamide ethanolamine salt [79]. The authors studied the effects of niclosamide ethanolamine salt on human liver carcinoma cells and in mouse livers and found that it increased lipid oxidation and stimulated the AMP-activated protein kinase (AMPK), which can phosphorylate and inhibit acetyl-CoA carboxylase 1, an inhibitor of mitochondrial β -oxidation.

6. Niclosamide and artery constriction

Arterial vasoconstriction is the dynamic narrowing of the blood artery vessels in response to signals [81]. Li et al. reported that treatment of rats with a more water soluble form of niclosamide, niclosamide ethanolamine, relaxed phenylephrine- and high K^+ (KPSS)-induced vasoconstriction, and that pre-treatment with niclosamide ethanolamine inhibited phenylephrine- and KPSS-induced constriction of rat mesenteric arteries. Due to its mitochondrial uncoupling activity, niclosamide ethanolamine reduced the cellular ATP/ADP ratio in vascular smooth muscle cells and activated AMP-activated protein kinase (AMPK) activity in smooth muscle cells and rat thoracic aorta. Niclosamide ethanolamine treatment increased cytosolic $[Ca^{2+}]_i$ and depolarized mitochondrial membranes in vascular smooth muscle cells [82]. These results suggest that niclosamide has potential as an anti-hypertensive drug.

7. Niclosamide and endometriosis

Endometriosis is an estrogen-dependent gynecologic disease that results when tissue that normally grows inside the uterus grows outside in the peritoneum instead [83]. It affects about 6–10% of women of reproductive age and is without effective pharmacotherapy. Prather et al. reported that niclosamide reduces the size of endometriotic implants in a mouse model of endometriosis through inhibition of cell proliferation and inflammatory signaling pathways, including RelA (NF- κ B) and STAT3 activation, without disrupting reproductive function in female mice [84].

8. Niclosamide and neuropathic pain

Neuropathic pain is a pathological condition affecting about 6–8% of the population worldwide where chronic pain emanates from damaged or diseased somatosensory nerves. There are few effective therapies [85]. Ai et al. reported that niclosamide is a low-nanomolar allosteric antagonist of Group I metabotropic glutamate G protein-coupled receptors (mGluRs), with high selectivity for Group I over homologous Group III mGluRs. Preclinical data demonstrated that in a mechanical hyperalgesia model of neuropathic pain in rats, pain-related behavior is reversed by niclosamide treatment [86]. Zhang et al. reported that Wnt signalling underlies pathogenesis of neuropathic pain. Both Niclosamide and an inhibitor of Wnt release (IWR) were effective in two rodent pain models [87].

9. Niclosamide and rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory autoimmune disease that may result in synovial inflammation, hyperplasia of synovial tissues, and joint damage. There are no effective therapies targeting the causes of rheumatoid arthritis, just non-specific anti-inflammatory treatments to alleviate symptoms [88]. Liang et al. reported that niclosamide reduced cytokine expression and release from TNF- α -induced human rheumatoid arthritis fibroblast-like synoviocytes. Niclosamide treatment inhibited serum-induced synoviocyte migration and invasion, and produced alterations in the filamentous-actin cytos-

keletal network in these cells. Niclosamide decreased TNF- α -stimulated MAP kinase and IKK/NF- κ B signalling activity in synoviocytes. In addition, niclosamide treatment reduced the severity of injury in the collagen-induced arthritis mouse model [89]. Huang et al. also reported that niclosamide induces apoptosis in human rheumatoid arthritis-derived fibroblast-like synoviocytes [90].

10. Niclosamide and Sclerodermatous graft-versus-host disease

Graft-versus-host disease may occur after a bone marrow or stem cell transplant in which patients receive stem cells from a donor, and these cells attack host tissues and organs as foreign. This disease is a leading cause of morbidity and mortality after such transplant [91]. Morin et al. reported that niclosamide treatment provided beneficial immunological effects and reversed clinical symptoms of graft-versus-host disease, including alopecia, vasculitis, and diarrhea, and also prevented fibrosis of the skin and visceral organs in the sclerodermatous graft-versus-host disease model using BALB/c mice provoked by B10.D2 bone marrow and spleen cell transplantation [92]. The beneficial effects of niclosamide were associated with inhibition of STAT3, Wnt/ β -catenin, ERK 1/2, AKT, and Notch signaling pathways in earlobe skin of mice.

11. Niclosamide and systemic sclerosis

Systemic sclerosis is a connective tissue disorder characterized by fibrosis of the skin and internal organs, vascular alterations, and dysimmunity including the presence of autoantibodies to nuclear proteins, all without a defined pathological cause [93]. Morin et al. reported that niclosamide treatment led to an improvement of the disease in a mouse model of systemic sclerosis induced by hypochlorous acid. Niclosamide-induced inhibition of STAT3, AKT, and Wnt/ β -catenin pathways were observed [94].

12. Niclosamide's multi-functional activity and mechanisms of action

Identifying unifying mechanisms underlying niclosamide's pleotropic biological activities is difficult due to gaps in our knowledge of targets that interact directly with niclosamide. For many of the activities reported within it is unclear if a specific interaction with a biological target molecule drives the observed result, if an indirect mechanism is operating, or if combinations of both occur. Most of the studies do not address this mechanistic issue. Thus no direct binding interaction between niclosamide and a distinct biological target molecule has been established to account for the reported impact on signaling pathways or biological observations cited within (Tables 1 and 2). Niclosamide's ability to act as a protonophore, uncouple oxidative phosphorylation, or affect pH balance in some cells has been proposed as underlying indirect mechanisms to account for Niclosamide's activity against helminths, activity against mTORC1, activity in mouse models of Type 2 diabetes and fatty liver disease, activity against bacteria and viruses, and activity in antihypertension models. Given niclosamide's ability to inhibit signal transduction pathways that drive the transcription of multiple gene products, it is likely that some of niclosamide's reported biological activities may result from cross-talk between signaling pathways [95–100].

The chemical structure of niclosamide contains structural features associated with pleotropic pharmacologic activity. Niclosamide is a member of the salicylanilide class of pharmacologic agents and is a derivative of salicylic acid. Imbedded within these classes and within niclosamide is an aryl β -hydroxy-carbonyl pharmacophore motif. This structural motif is resident in a large number of diverse biological natural products isolated from plants, fungus and bacteria, and it is resident in multiple approved medicines across a variety of therapeutic categories. Representative examples of pharmacologic agents contain-

Table 2
Summary of signaling pathways and biological processes in disease models cited within affected by niclosamide.

Pathway or process affected by Niclosamide	Tape worm	Cancer	Bacteria	Virus	Metabolic syndrome	Artery constriction	Endo-metriosis	Neuro-pathic pain	Rheumatoid arthritis	Graft-versus-host disease	Systemic sclerosis
Uncoupling of oxidative phosphorylation	4–7	9			79	82					
Wnt		9, 11, 19, 23–25, 29, 35, 44, 45, 55						87		92	94
mTORC1		13, 29									
STAT3		14, 15, 31, 37, 38, 50					84			92	94
NF-κB		29, 34, 35					84		89		
Notch		26, 29, 33								92	
AKT/ERK/Src		20								92	94
AR-V7		49									
C-Fos, C-Jun, E2F1, c-Myc		41									
mGluRs								86			
Metabolic pathways		42									
ROS		34									
Mitochondria		52, 55				82					
pH	78	13, 52	58	70							

ing this motif are salicylic acid, diflunisal, aminosalicic acid, antimycin, balanol, mycophenolate, flavonoids, doxycycline, daunorubicin, and eticlopride. In many of these examples a direct binding target and mechanism has been identified. Given the presence of this structural motif, it is not surprising that niclosamide has pleotropic biological activities and has the potential to interact with multiple biological targets. More research is needed to define the structure-activity relationships of niclosamide and the biological targets to which it binds in order to identify more selective agents and define underlying mechanisms. Toward this end, recent structure-activity studies have demonstrated that niclosamide's effects on ATP homeostasis can be separated from its effect on Wnt signaling [101].

13. Pharmacokinetic improvement of niclosamide for treating systemic disease

Niclosamide is a monohydrate that dehydrates above 50 °C and melts at 232.2 ± 0.2 °C, with a heat of fusion of 40.7 ± 6.5 kJ/mol. Its LogD at pH = 7 is 4.48 and it is essentially insoluble in water [102]. Niclosamide has low oral toxicity in mammals, and an oral median lethal dose (LD₅₀) in rats of > 5000 mg/kg [103,104].

Chang et al. reported the pharmacokinetic parameters of niclosamide in the rat when administered orally at 5 mg/kg [105]. Niclosamide exhibits a short half-life (6.0 ± 0.8 h). Niclosamide was rapidly absorbed with a T_{max} of less than 30 min. The C_{max} is 354 ± 152 ng/mL. AUC and bioavailability were 429 ± 100 and 10%, respectively. Osada et al. reported the pharmacokinetic parameters in mice orally dosed at 200 mg/kg, and observed a similar kinetic profile [24]. They also demonstrated that niclosamide concentrations in tumor tissue (37 ng/g tissue) were similar to those in plasma (38 ng/mL) measured at 24 h after the final administration, a concentration well-below the IC₅₀ of niclosamide *in vitro* in Wnt signaling and in cell growth assays [24].

For treatment of systemic diseases, efforts to improve systemic exposure to drug have been focused on employing nanotechnology and pro-drug approaches. Ye et al. used a wet media milling technique to prepare niclosamide nanocrystals approximately 235 nm in size [106]. However, this nanocrystal formulation showed no significant improvement in plasma concentration vs. time profiles between nanocrystals and control niclosamide when administered intravenously (i.v.) to rats, though an increased tissue concentration was observed at 2 h. Lin et al. reported that by using single-capillary electrospray method, they developed a water-soluble form of nano-niclosamide. The plasma

concentration of niclosamide in this nano-formulation, via both oral and IV administration, peaked right after the distribution phase at 4 h as previously reported [24,107].

Recently, our group reported that an acyl derivative of niclosamide, DK-520, significantly increased both the plasma concentration and the duration of exposure to niclosamide when dosed orally [108]. This is the first report to successfully increase the systemic drug exposure of niclosamide in plasma and to extend its duration of exposure. In order to make more effective use of niclosamide, additional work needs to be done to improve its solubility, absorption and systemic bioavailability.

14. Conclusions

Beyond its approved medical use for parasitic disease treatment, niclosamide has demonstrated preclinical activity in many disease models, ranging from cancer and metabolic diseases to multiple types of infections (Table 2). Currently there are four clinical trials of niclosamide in colon cancer and prostate cancer in the ClinicalTrials.gov clinical trials registry. Others will surely follow as the beneficial effects of niclosamide are appreciated in specific diseases. Improvement of the pharmacological and pharmacokinetic properties of niclosamide through re-formulation or pro-drug strategies are approaches to make more widespread use of this drug. The development of novel niclosamide derivatives that are biased toward targeting specific signaling pathways or biological functions in specific systemic diseases is a second approach to make use of the remarkable power of niclosamide.

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