Medicinal Significance of Nitroimidazoles — Some Recent Advances

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Nitroimidazoles have therapeutic uses as anaerobic antibacterials and antiprotozoal agents. Recently, they have been found to possess other interesting biological activities of therapeutic potential such as, radiosensitizers in treatment of cancer, control of fertility, and antitubercular therapy. Various aspects on the chemistry and modes of action of nitroimidazoles are discussed.

Keywords: Nitroimidazoles, Therapeutic uses of nitroimidazoles, Biological activities

Introduction

Medicinal chemists have often been skeptical about the use of nitro group in potential medicinal agents because certain untoward observations have been made with regard to the nitrocompounds. Compounds like trinitrotoluene, employed in explosives arsenals, cause methemoglobinemia following skin absorption, and picric acid useful as antiseptic, turns out to be a systemic poison. Chloramphenicol, an antibacterial discovered in 1948, was found to have a nitrophenyllsystem. Since then, other nitroheterocycles have been discovered among antimicrobial agents and have been used in severe typhoid and other salmonellal infections. The discovery of azomycin (1), a 2-nitroimidazole, by Nakamura1 and demonstration of its antibacterial antitrichomonal properties2, revolutionised the area of nitroimidazoles. Azomycin became the chemical lead for extensive synthetic development of many other compounds. This resulted in the emergence of a compound now known as metronidazole (2), which was introduced as a chemotherapeutic agent in 1960 in Europe and in 1963 in the US. It became a drug of choice in the treatment of several protozoal infections and infections caused by obligate anaerobes. The present communication reviews the development of nitroimidazoles as medicinal agents with their current status not only as antimicrobials but having other interesting biological profiles, discovered recently.

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\begin{align*}
&\text{Azomycin (1)} \\
&\text{Metronidazole (2)}
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Classification of Nitroimidazoles

Depending on the positions available in imidazole heterocycle, nitroimidazoles have been classified as 2-nitro, 4-nitro-, and 5-nitroimidazoles. Out of them, 5-nitroimidazoles have established themselves as significant therapeutic agents with antiprotozoal and antibacterial properties.

2-Nitroimidazoles

Azomycin (1) discovered from the extract of soil Streptomyces cultures was the first 2-nitroimidazole to exhibit activity against several protozoans, specifically trichomonads. It provided a prototype for the development of other nitroimidazoles, such as misonidazole (3), which showed activity against intestinal amebiasis in rats similar to that of metronidazole3,4. Misonidazole also find therapeutic use in the treatment of neoplastic diseases5. Benznidazol (4) is useful in the treatment of South American trypanosomiasis (Chagas’ disease) due to...
infection with Trypanosoma cruzi, especially in early acute stage of the disease.

4-Nitroimidazoles

As such, there appears to be no report on the antiprotozoal or antibacterial effects of 4-nitroimidazole derivatives. However, a drug azathioprine (5) which has 4-nitroimidazole-5-yl moiety attached to purine through sulphur has shown promise as an immunosuppressant.

5-Nitroimidazoles

5-Nitroimidazoles constitute the largest group of nitroimidazoles with antiprotozoal and antibacterial activities. Metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (2) is one of the most extensively used drug in the treatment of trichomoniasis, amoebiasis, and giardiasis and shown to be active against anaerobic bacteria. It is the most active agent available against obligate anaerobes and is of major value in the treatment of serious infections due to these organisms. Metronidazole has been a subject of extensive chemical modifications to improve its bioavailability.

Following the success with metronidazole, a hectic activity of synthesising and testing other 5-nitroimidazole was initiated. As the 1-(2-hydroxyethyl) side chain at position 1 of metronidazole is readily oxidised metabolically, replacement of this side chain resulted in the development of other clinically useful antibacterial and antiprotozoal drugs, like tinidazole (6), panidazole (7), ornidazole (8), secnidazole (9), carnidazole (10), and ternidazole (11).
Mechanism of Antimicrobial Actions of 5-Nitroimidazoles

The mechanism of action of nitroimidazoles has been studied in detail. The nitroimidazoles are selectively toxic to anaerobic or microaerophilic microorganisms and for anoxic or hypoxic cells. The 5-nitroimidazoles, as represented by metronidazole, require metabolic activation by sensitive organisms for their antimicrobial effect. After the drug enters into the cell the nitro group of the imidazole heterocycle accepts electrons from the electron transport proteins with sufficiently low negative redox potential such as, flavoproteins including xanthine oxidase, NADPH cyt P450 reductase and NADPH cyt c reductase in mammalian cells and ferredoxin or their equivalent, such as pyruvate dehydrogenase ferredoxin oxidoreductase in protozoa or bacteria. The rate of intracellular reduction is important because it determines the rate of drug uptake into the cell via a concentration gradient. The complex reduction is catalysed by nitroreductase in mammalian cell and by iron-sulphur complexes in other case. The electrons are supplied by either reduced nicotinamide adenine dinucleotide phosphate (NADPH) or sulhide. The reduction of nitroimidazoles is a complex process involving sequential addition of an electron, protonation and disprotonation, as shown in Figure 1. The chemically reactive intermediates, formed during the electron reduction of the nitro group to the corresponding hydroxylamine, account for the antimicrobial activity.

![Figure 1 — Bioreduction of nitroimidazoles.](image)

A new 5-nitroimidazole fexinidazole (HOE 239) (19) with a 4-methylthio- phenoxy methyl group at position 2 was found to be highly active against cure experimental murine CNS-trypanosomiasis with a 1 d treatment, when given in combination with melarsoprol. Sulphimidazole (20) is the most recent compound in which a p-aminobenzene-sulphonamide moiety has been attached at position 2 of the 5-nitroimidazole ring. It possesses a useful spectrum of activity.

![Fexinidazole (19)](image)

![Sulphimidazole (20)](image)
of nitroimidazoles. The exact process by which these intermediates destroy the cells has not been elucidated but it has been suggested that these intermediates react with cellular macromolecules, such as deoxyribonucleic acid (DNA), proteins, and membranes. The entities which are cytotoxic and cause DNA damage are nitro-free radical and the superoxide anion, formed by its reaction with oxygen, as well as the products of further reduction of nitro group [i.e. nitroso (R-NO), nitroso-free radical (R-NO)] or hydroxylamine (R-NHOH).

**Pharmacokinetics**

The pharmacokinetics of nitroimidazoles, including that of metronidazole, has been studied intensively. They are readily absorbed following administration by mouth with excellent degrees of bioavailability. Peak plasma concentration of approximately 5 and 10 µg/mL are achieved usually within 1 to 2 h of single dose administration of 250 and 500 mg, respectively, of metronidazole. Nitroimidazoles get widely distributed in the body. Metronidazole is reported to appear in most of body tissues and fluids, like bile, bone, breast milk, cerebrospinal fluid, seminal fluids, and vaginal secretions.

Metronidazole gets metabolized by the liver through side chain oxidation and glucuronide formation. The principal oxidation metabolites are 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole which also have antibacterial activity. It is detectable in plasma and urine. The other major metabolite which has no antibacterial activity is 2-methyl-5-nitroimidazole-1-acetic acid. Small amounts of reduced acetamide and N-(2-hydroxyethyl)oxamic acid (HOA) have also been detected in urine.

The half-lives of 5-nitroimidazoles vary from 8-20 h. The values reported are metronidazole (8 h), ornidazole (12–14 h), benznidazole (10.5–13.6 h), and secnidazole (20 h).

**Recent Advances in Other Biological Profiles**

Besides having antiprotozoal and antibacterial activity, nitroimidazoles have exhibited other interesting biological activities, which are useful in therapeutics. These are nitroimidazoles as radiosensitizers, antifertility potential of nitroimidazoles, and antitubercular profile of certain nitrimidazopyrans.

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**Nitroimidazoles as Radiosensitizers in the Treatment of Cancer**

The hypoxic cells present in most of the solid tumours, limit the successful local control of these tumours by radiotherapy of growth, essentially outstripping the tumour's vascular system, hence reducing the supply of essential nutrients, particularly oxygen. However, tissue oxygen tension decreases with distance from the capillary and gradually falls to a level insufficient for cell division. Eventually the oxygen deprived cells die and this causes the local necrosis observed in most solid tumours. A viable hypoxic cell, which occurs in the interface regions between the well-oxygenated tissue and the necrotic regions, are radiation resistant relative to oxic cells. So the radiation resistance is the largest factor influencing local tumour control by radiation.

In the early 1970s, researchers in Canada and England, discovered that nitrofurans and nitroimidazoles are radiosensitizers of hypoxic (oxygen deficient) cells. Cellular response to ionizing radiation is strongly influenced by oxygen concentration; hypoxic cells are much more resistant to radiation damage than are normally oxygenated cells. The sensitizing effect of molecular oxygen is probably a consequence of the rapid chemical reactions between oxygen and short-lived free radicals generated by radiation absorption. It has been suggested that electron affinic (or easily reduced) chemicals might mimic the sensitizing action of oxygen and thereby overcome the radiosensitivity of hypoxic cells. The discovery of the radio sensitizing properties of nitro-heterocycles led to the intense interest in the medical application of radiosensitizers.

The correlation between one electron reduction potential and ability of nitro compounds to act as radiosensitizers of hypoxic cells is central to the development of agents useful as adjuncts in radiotherapy. The first electron affinic sensitizer based on the nitro functional group to be tested...
clinically was metronidazole that was already in clinical use as an anti-trichomonal agent. The lead compound, however, in this series was 2-nitromidazole. Misonidazole, extensively investigated clinically as radiosensitizer. The neurological toxicity of misonidazole is the major limitation in its application. It has been shown that the neurotoxic property is due to lipophilic character. In an effort to reduce this side effect, less lipophilic 2-nitroimidazoles, such as desmethylmisonidazole and SR-2508 were synthesized which showed less neurotoxicity than misonidazole in keeping with their lower lipophilicity. A Roche compound R003-8799 which has a basic side chain has been found to be superior to misonidazole with improved tumour uptake. The improved uptake has been attributed to the acid-base properties of the piperidine group in the side chain structure. During the development of the chemosensitizers, numerous compounds have been synthesized which were substantially more efficient in vitro than would be predicted on the basis of the electron-affinity relationship. This is particularly true of some compounds that react efficiently with intracellular glutathione. Most of these compounds show little, if any, activity in vivo. More recently, derivatives of misonidazole which contain in a side chain a monofunctional alkylating groups, such as aziridine, have been prepared. A lead compound in this series is RSU 1069, chemically 1-(2-nitro-1-imidazolyl)-3-(1-aziridinyl)-2-propanol. It has been found to be more efficient radiosensitizer than misonidazole, both in vitro and in vivo. However, gastrointestinal toxicity limits the dose than can be administered clinically to levels that would be expected to provide a benefit in cancer therapy. RB 6145 is a prodrug of RSU1069 that has lower toxicity with similar potency.

Binding of Nitroimidazoles to Hypoxic Cells — Use in Detection of Cancer

Nitroimidazoles are reduced under hypoxic conditions to intermediates that bind cellular macromolecules. This has been exploited in the detection of tumour hypoxia. Early studies utilized 14C-labelled misonidazole and autoradiography, and served to demonstrate that hypoxia does occur in patient’s tumours. Another approach has been to use nitroimidazole derivatives that can be detected immunohistochemically. Fluorinated nitroimidazoles have been synthesized for use in non-invasive detection of hypoxia.

Chemosensitization

Nitroimidazoles potentiate some chemotherapeutic agents, particularly alkylating agents. Hypoxia is a prerequisite for chemosensitization, which gives it some specificity for tumours. Three mechanisms, which have been suggested to account for the chemosensitization and observed experimentally are depletion of intracellular thiols; modification of pharmacokinetics, and increased cross-linking of DNA.

Antifertility Potential of Nitroimidazoles

Ornidazole, 1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole has been found to be an effective antifertility agent in male rats only. It acts rapidly and reversibly to decrease the motility of distal epididymal spermatozoa rather than on major epididymal secretions. It does not prevent access of spermatozoa to eggs in the oviduct while inhibiting fertilization, and reduces the activities of two glycolytic enzymes of epididymal spermatozoa without reducing the ability of sperms to undergo capacitation and acrosomal loss in vitro. Ornidazole is used clinically for the treatment of genital tract infections in both women and men and thus has some potential as a basis for human contraceptive agents. In contrast, metronidazole is reported to take 6wk to reduce spermatogenesis and render male rats infertile, as a result of azoospermia.
Some observations with regard to structure activity relationship for antifertility potential have been drawn. Of the three functional groups attached to the imidazole ring of ornidazole, viz. methyl, nitro, and (chlorohydroxy)propyl, the three-carbon side chain at position 1 is essential for antifertility action. The compounds that lack this side chain, such as 2-methyl-5-nitroimidazole or those having side chains of one carbon, such as dimetridazole or of two carbons with either a hydroxy group, such as metronidazole or chlorine, such as 1-(2-chloroethyl)-2-methyl-5-nitroimidazole are all inactive. Even when the side chain has three-carbon, such as ornidazole, but lacks chloro and hydroxy functions, such as 1-(2,3-epoxy)propyl-2-methyl-5-nitroimidazole or which has a longer side chain, such as timidazole has no effect on fertility.

**Antitubercular Profile of Certain Nitroimidazopyrans**

A series of bicyclic nitroimidazofurans, originally investigated as radiosensizitors for use in cancer chemotherapy, were found to possess activity against cultured replicating *Mycobacterium tuberculosis* (MTB) and had significant in vivo activity in a murine infection model. The lead compound in this series, CGI-17341 was mutagenic, discouraging further investigation of the antibacterial activity of the compound series. These studies however, suggested, that the bicycic nitroimidazoles might be potential antitubercular agents. A series of over three hundred 3-substituted nitroimidazopyrans (NAPs) were synthesized on the basis of the structure of CGI-17341. Over 100 of these compounds possessed substantial antitubercular activity, matching or exceeding that of CGI-17341.

The active compounds lacked the mutagenicity. Structure activity relationship studies focusing on antitubercular activity revealed substantial variety in the tolerated substituents at C3, but optimal activity was achieved with lipophilic groups. The stereochemistry at C3 was important for activity, as the S-enantiomers were generally at least 10-fold more active than the *R*-enantiomers, NAP activity was found to be highly specific for the MTB complex, and showed only modest or no activity against mycobacteria outside the MTB complex (*M. avium, M. smegmatis, M. chelonae and M. fortuitum*).

Over 50 NAP compounds with demonstrated activity against cultured MTB were screened for in vivo antitubercular activity, using a short-term murine model of infection with an MTB reporter strain expressing firefly luciferase (rMTB-lux). Although PA-824 was not the most potent NAP against cultured MTB clinical isolates, it was found to be most active in infected mice when orally administered at 25 mg/kg. This indicated that PA-824 might possess more desirable pharmacokinetic properties than the other more potent NAP compounds that were tested.

The activity of PA-824 against both multidrug-resistant and non-replicating microaerophilic MTB suggested that the NAPs act by a new mechanism. Studies on the macromolecular effects of PA-824 on MTB revealed that both protein and lipid synthesis were substantially inhibited at drug concentrations and time intervals when nucleic acid synthesis was unaffected. Lipid synthesis was not affected by treatment with amikacin, an aminoglycoside protein synthesis inhibitor, indicating that the effect on lipid synthesis is specific and not a consequence protein synthesis inhibition in MTB. As demonstrated by PA-824, culture and models of animal infection the NAPs possess bactericidal activity against MTB comparable to that of isonazid at doses that are safe in mice and guinea pig. PA-824 and the NAPs are also small, orally active molecules that should be amenable to large-scale production, making them practical and attractive candidates for the clinical development of a new antitubercular therapy. In addition, activity against multidrug-resistant tuberculosis and demonstrable activity by PA-824 against static MTB could offer significant potential for reducing the duration of therapy over current antitubercular therapies that target only growing organisms.

**References**

5 Singh H & Kapoor V K, Medicinal and pharmaceutical chemistry (Vallabh Prakashan, Delhi) 1996, p. 419.