Review



# Chemical Versatility of Halofuginone; Its Detoxification and Future Pharmacotherapeutic Potentials

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*Review*

# Chemical Versatility of Halofuginone; Its Detoxification and Future Pharmacotherapeutic Potentials

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**Abstract:** Cancer is a lethal disease worldwide that damages bodily tissues, linked with predisposing variables including inheritance, social, infections and curative measures requiring behavioral modifications. But now a days, researchers are working to find targeted drug therapy with limited side effects that are associated with previous approaches. Plant based novel drug Halofuginone (HaF), by blocking prolyl tRNA synthetase exhibits promising diverse clinical activities, against fibrosis, cancer, metabolic, and infectious disorders. However, the drug is quite neurotoxic and gastrotoxic requiring thorough investigational analyses as per preclinical studies at Phase-II clinical trials. The present study explored various databases, software and monographs to establish therapeutic aspects of this drug to address gaps by highlighting the potential impact of salt reformation on improving its properties. Hence, our study invites the researchers to look upon novel Halofuginone derivatives with other possible halogen salts like HCl to enhance pharmacokinetics and pharmacodynamics qualities of Halofuginone. Our present study summaries the possibilities of other form of Halofuginone for safe utility in pharmacotherapeutics for future research in this area, provided its toxicity concerns are adequately addressed through innovative strategies.

**Keywords:** Immunosurveillance and immunomodulation; NFK-B- P60/P65; Prolyl-transfer-RNA-synthetase; TGFβ-SMAD3 / AKT-MTOR; PI3K/Akt-Bax/Bcl2; *Plasmodium falciparum;* Tumorigenesis ; Cefotiam HCl and,, Exidartinib HCl; Cytokine IL-17; p38-MAP-Kinase / P-ARP

## 1. Introduction

Cancer (Canc.) is a life threatening ailment with various predisposing factors mainly socioenvironmental, genetic, infectious diseases may be prevented through behavioural changes or vaccination to prevent infection [1]. Canc. hallmarks include evading growth suppressors, sustaining cell proliferative signalling events, tissue invasion, metastasis, angiogenesis or neo-vasculature, cell death/apoptosis (Apo.), mutated energy metabolism, and loss of immunosurveillance [2,3]. In 2018 around 18.1M new cases were diagnosed and are expected to rise to 21.6M by 2030 annually, and in 2020 nearly 10M deaths or approx. 1 in every 6 deaths or approx. 1 in every 6 deaths reported [4,5].

Global burden and prevalence were expected to increase up to 27.5M with 16.3M death rate or approx. 1 in every 6 affected by 2040 [5,6]. As per US data of 2022 new Canc. patients count was 1.9M out of which 36.33 % deaths are projected [1]. Around 46.5K Canc. deaths and 176.6K new cases were projected among the Hispanic population in the U.S. and Hawaii in 2021 accounting for 20% of deaths [1]. During lifetime, as per one estimate out of 100, about 39 women and 40 men will likely develop Canc. and may differ depending upon factors like genetics, cigarette smokers, illicit drug users and workers expose to radiations [1,7].

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Prevalence varies based on organs affected such as breast Canc. 2.26M, lung Canc. 2.21M, gastric Canc. (GC) 1.089M, hepatic Canc. (Hep.ca) 0.96 M and colon Canc. by 1.93M [8]. Deadliest Canc. reported in Asia include lung, breast, GIT and liver [6]. With the highest population ratio across the globe (60%), life expectation in Asia has increased and was expected to be around 25% more by 2050 among people aged ≥60yrs [9–11]. Most common Canc. in young females are 13.8% lung, 10.8% breast, 10.6% colorectal, 8.6% stomach and 6.9% liver with recommendation of intensive early Canc. diagnosis, precautionary measures, and lifestyle modifications. In year 2020, 950,3710 new cases are reported with age-standardized rate of incidence was 0.169.1 % for both sexes with highest in Japan, Korea and Israel and total of 580,94,31 deaths, most commonly lung Canc. 19.2% with liver ranked at the second 0.1016% (highest in Mongolia, China and Armenia). More in males around 0.1852 %, highest lung then G.I.T, whereas for females, 0.1567 % with highest of breast then colorectal and lung. Breast Canc. in 2004-2013, rise amongst Asia-Pacific females (20-49 years) due to reproductive factors, high levels of breast exposure to oestrogen at menopause with aging, over-diagnosis of thyroid and lung Canc. with tobacco use [12–20]. GIT Canc. -related deaths were because of higher *H. pylori* level [9,21]. In Korea, more prevalent was Canc. of thyroid 27.5% during 1999-2018, and more in Japan 1985- 2010 while lesser in the Philippines and Israel in both sexes [22–27]. Canc. prevalence increased in Pakistan due to smog, population and pollution, with 19M new cases in 2020 [8].

More than 60 % clinically proven natural origin anticancer drugs are available and research and NDDA are focused on diversity and structural modification, the main foundation of new anticancer drugs [28]. Among these novel approaches, one of the plant origin drugs Halofuginone (HaF), could be a medical breakthrough as per its multiple mode of actions and associated efficacy in Canc. and non-cancerous diseases. However, the drug is in phase 2 trials because of toxicity. Through our review we will try to elucidate its mode of action, SAR, multiple targets and how we can enhance its physicochemical parameters for Canc. therapy in future for the betterment of mankind.

## 2. Materials and Methods

The author searched systematically, thoroughly and screen with comprehension most relevant, authentic and original group of potential studies, index, articles and writings from the Scientific literature; Scopus; Uniport; RSCB; PD; WOS; JSTOR; CNKI DATABASE; books; Electronic DATABASES; and Google Scholar; PubMed; Science Direct; Elsevier; National library of medicine Bethesda; Maryland; NCBI; The New York Academy Of Medicine; Library of Speech Rehabilitation; NY; ST. Thomas 's Hospital Library; Repository of Kings College London; Oxford Academic Repository; Wiley Online Library; CAS data base; PubChem; American Canc. society; IJCEP; MDP; journals including Chinese Chemical Letters; Journal of Medicinal Chemistry; Journal of Biochemistry; Sage journals from February 2023 till June 2024. Moreover, Software's that are manifested include Adobe Photoshop 2021; Molinspiration bioactivity score v-2022.08; Chem 3D Pro 12.0 for pictorial explanations and literature review writing. We have set predetermined rules for inclusion and exclusion of applicable studies from primary studies on HaF with significant investment of time in order to ensure objectivity, free from biasness with multiple time screening to ensure high scientific quality of the selected studies, with relevance to problem of interest and maximum validity. For this purpose, we collect, precise, combined, systematically organize data and compare the evidence extracted HaF data and present it in an eloquent way and in future recommends other researchers. In order to meet set criteria and to make sense of existing knowledge on a problem of interest in our case HaF toxicity, we provided a comprehensible lens from each angle, articles included are from 1946 till 2024. Total 414 research and review articles are explored and studied for this review altogether to identify, critically analyse and detailing of all relevant literature on a Canc. in correlation to HaF, in order to derive conclusions about the mechanism of actions on various molecular genetic factors in cancerous and noncancerous diseases, currently available or under study analogues of HaF and identifying gaps in previous literature available.

### *2.1. Biochemistry and Structural Analogues of Halofuginone:*

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In advanced anticancer research, folk Chinese medicines particularly of natural origin (including plant, animal, fungi) have been considered promising. Among these plant origin drugs novel RNA synthesis inhibitors agents, a structural derivative of Febrifugine (Febr.) with IUPAC name 3-[3-[(2R,3S)-3-hydroxypiperidin-2-yl]-2-oxopropyl]quinazolin-4-one is Halofuginone (HaF) with IUPAC name 7-Bromo-6-chloro-3-[3-[(2S,3R)-3-hydroxy-2-piperidinyl]-2-oxopropyl]-4-quinazolinone and has been in use for over 2000 years, an analogue of quinazolinone alkaloid extracted from leaves and roots of Chinese herb '*Dichroa febrifuga*' Hydrangeaceae family in 1948, used as an antimalarial medicine and in poultry feed and also received US-FDA certificate in early 1980s [29–31]. In 1967 Waletzky et al. developed a compound utilized in poultry feed as a coccidiostat in cattle and show positive outcomes for treatment of fibrotic diseases, immunosuppressive disorder like cancers, and excessive immunostimulant disorder like autoimmune diseases named as HaF **(Figure 1C)** [32-37]. Quinazolinone ring containing alkaloids [32–37includes Febrifugine (2R,3S) **(Figure 1A)**, Isofebrifugine (2S,3S) (stereoisomer of Febrifugine) **(Figure 1B)** also isolated from Dichroa Febrifuga, were reported along with their skeletal structures in 1967, however absolute configuration was attained after more than 40yrs of research, and another derivative Halofuginone hydrobromide discovered and synthesized by American Cyanamid Company, later on transferred to Roussel Uclaf S and under the named "Stenorol" A. who marketed it in a premix [38–41]. Following the first isolation test back in 1946 Febr. shown markedly higher activity both in vitro and in vivo than quinine, or chloroquine against several strains of malaria that was why gathered a lot of attention [38,42–44]. However, early findings from human trials exposed that it has several side-effects including nausea, vomiting, and lethargy resulted in creation of several analogues with significantly enhanced activity and metabolic stability, against Canc. as well [45].

Febr. structure consist of three parts: the ketone linker, the piperidine ring, and the quinazolinone ring **(Figure 1D)**. To provide better therapeutic candidate all parts of Febr. structure have been altered. Several metabolites were detected in 2003 in mouse model and isolated [46]. Alteration in the quinazolinone ring was done by Baker et al. including mono and di substitution at the 5th, 6th , 7th, and 8th positions, showing better anticancer efficacy, by mono substitution at the 5th and 6th position and di substitution at the 6th and 7th positions against *Plasmodium lophurae* in ducks and similarly, further modification was done by increasing the range of substituents and altering the attached type of aromatic ring as well [47–49]. The oxidised quinazolinone ring derivatives **(Figure 1E)** have shown reduced anti-malarial activity against P. falciparum, by blocking the susceptible positions and by changing the oxidation potential of the ring making the quinazolinone group as an attractive target for modification due to metabolic susceptibility[48–55].

Shingo Hirai and Haruhisa Kikuchi, coresearchers studied and observed the Febr. analogues with the aim of conserving the strong antimalarial activity and potency in past, as it can be valuable leads for new drugs development (NDD) and for studying other related therapeutic properties such as anticancer or for scleroderma treatment. Basicity of both the nitrogen atoms at position 1 and the 1″ of Febr. was important in conversing powerful antimalarial activity in vitro and the metabolite 4 given below **(Figure 3)**, of Febr. with hydroxy '-OH' substitution at position C-6 in place of 'H' at quinazolinone ring, exhibited powerful antimalarial activities. Initial data suggest that the 'OH' group has shown low toxicity against mammalian cells and preserves antimalarial activity against P. falciparum, seems to be viable main component for the development of novel antimalarial drugs in future due to lesser side effects then parent compound [38,56]. The oral administration of this compound with ED50 values of 15mg/kg/day for 4 and 8mg/kg/day was seen to be effective against murine malaria and single administration at 3 mg/kg intraperitoneal in mice, has no associated toxicities, such as diarrhoea or weight loss, after four successive days of therapy [38–41,46,57].

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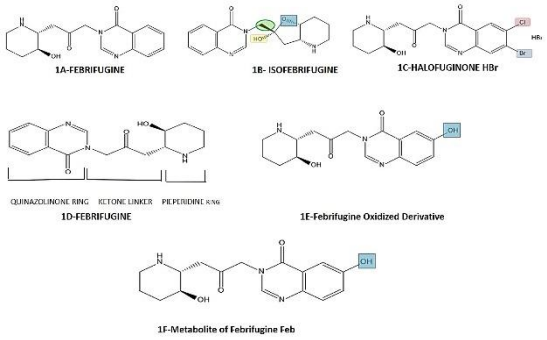


**Figure 1. (1A)** Structures of Febrifugine: (**1B**) Isofebrifugine stereoisomer of Febrifugine; **1(C)** Halofuginone Hydrobromide-halogenated derivative of Febrifugine with bromine at position 7 and chlorine at position 6 and HBr salt [38]; **(1D)** Structural Sections of Febrifugine[57]; **(1E)** Structures of Febrifugine Oxidized Derivative [46]; **(1F)** Structures of Metabolite of Febrifugine Febr-A(4) [46].

By organizational alterations at the quinazoline or piperidine groups It has been confirmed that majority of Febr. analogues have enhanced activity consisting of a modified or unmodified 4-quinazolinone moiety, but analogues with modification at the side chain attached to the $N_3$ of the 4-quinazolinone ring have no activity, additionally a synthetic racemic Febr. was about 50% as effective as natural Febr. Therefore, the absolute position of functional groups and quinazolinone ring, localisation of 'N' in piperidine and 'OH' are essential for the anti-malarial action in Haf therefore, approved by FDA for coccidiosis treatment in chickens and turkeys in the salt form with Hydrobromine. Haf-Lactate are also used for parasitic attack in cattle[58–62]. Many reports on its synthesis were published as per importance of Febr., along with study on its analogues, however early synthesis methods met various difficulties in relation to stereo-chemistry, complexity and could interconvert [32,39,62–64]. Among the derivatives of Febr. the IC50s of HaF were detected to be the best of all [63].

Modification of the piperidine ring during Febr. analogues manufacturing has also been considered, as studied by Baker et. al. studies he manufactured many isomers/analogues including Deoxy-Febrifugine 169 **(Figure 2A)**, the isomeric 4- hydroxypiperidine 170 **(Figure 2B)**, 5-hydroxypiperidine 171 **(Figure 2B)**, 3-hydroxymethylpyrrolidine 172 **(Figure 2C)**, and 4-hydroxymethylpyrrolidine 173 **(Figure 2C)**, without reporting their antimalarial activity [48–55,65–71]. However, Deoxyfebrifugine 169 antimalarial activity was reported by Takeuchi et al. in 1999 and synthesis was completed by Evans et al. in 2015 showing that it has reduced activity than Febr. against Plasmodium falciparum-FCR-3. after piperidine ring alteration, similar study was conducted by Cruickshank et al. in 1970 and Takeuchi by modification of piperidine analogues 175 **(Figure 2E)**, and the pyridine-based analogue 174 **(Figure 2D)** respectively, against *P. berghei* (in mice) and P. falciparum-FCR-3 (in vitro), in which the position of the 'N' in the piperidine ring has been altered leading to no activity [44,48–55,65–77]. Similarly, with a broad scope of modifications in a piperidine ring, a huge collection of analogues has been synthesised by Oshima et al., in parent compound Febr. Including, the substitution of 'N' and 'O' in analogue 176 **(Figure 2F)** with acetyl substitutions, resulting in reduced activity against Plasmodium falciparum-FCR-3, and similar result was seen in analogue 177 **(Figure 2G)** and analogues 178 **(Figure 2H)** synthesised by Zhu et al. having more activity against plasmodium sp. (chloroquine-resistant strain) and analogue 179 **(Figure 2H)** with change in the size and the optically activity of Pyrrolidine and Azepane analogues of the piperidine ring, when compared to Febr. [49,78,79].

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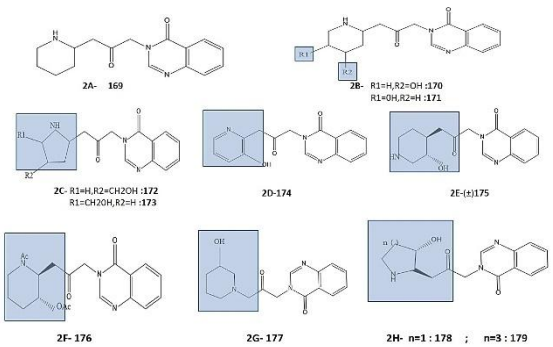


**Figure 2.** Structures of Metabolite of Febrifugine: Piperidine analogues **(2A)** 169- deoxyfebrifugine; **(2B)** 170- the isomeric 4- hydroxypiperidine; 171-5-hydroxypiperidine; **(2C)** Pyrrolidine analogues; 172-3-hydroxymethylpyrrolidine; 173 -4-hydroxymethylpyrrolidine; Piperidine analogues **(2D)** 174; pyridine analogue **(2E)** 175; Piperidine and pyrrolidine analogues; (**2F**) 176 with N- and O-protecting groups; **(2G)** 177; **(2H)** 178 and 179 with change in the size and the optically activity of Pyrrolidine and Azepane analogues of the piperidine ring [48–55,65–71,78–80].

However, some analogues showed similar activity to Febr. including analogues 180 **(Figure 3A)** and 181 **(Figure 3B)** against *P. falciparum* W2 and TM6 respectively [81–83]. Another researcher also discussed the importance of ketone linker, loss of functionality with the ketone elimination including analogue 182 **(Figure 3C)**, replacing it with amide including analogue 183 **(Figure 3D)**, acetone condensation analogue possessing a quinolizidine bicycle including analogue 184 **(Figure 3E)**, showing loss/decrease in activity against *Plasmodium falciparum*-FCR-3. Bicyclic compound "Febrifuginol" 185 **(Figure 3F)** and analogue 186 **(Figure 3G)** with a link to the 'OH' and 'N' of the piperidine ring, showed better activity and reduce toxicity respectively [48,58,79,84]. Fluoride containing analogue of Febr. was also synthesized and studied by Liu et al. namely 5-Fluorofebrifugine 187**(Figure 3H)**, with no reported data related to its impact on biological activity [85].

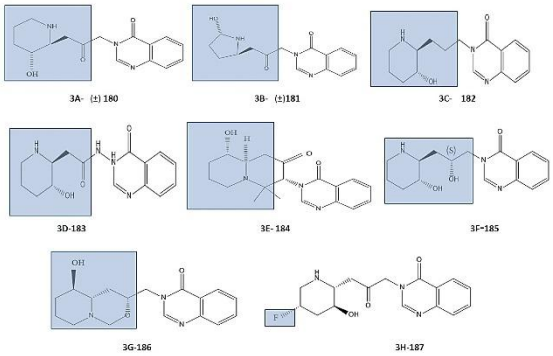


**Figure 3.** Structures of truncated Analog of Febrifugine: (**A)**, compound 180; (**B),** compound 181 [81–83]; Ketone Linker and fluorinated Analogues of Febrifugine; (**C),** Compound 182- loss of functionality with the removal; (**D)**, Compound 183- replacement with an amide; (**E)** Compound 184- acetone condensation; (**F)**, Compound 185 Bicyclic compound febrifuginol; (**G)**, Compound 86- with a linkage to the 'N' of the piperidine ring and the 'OH' group; (**H),** compound 187- fluorofebrifugine [48,58,79,84,85].

*2.2. Stereoisomeric Synthesis of Halofuginone HBr Salt:*

Synthesis for HBr Salt of HaF from Febr. precursor starts piperidine ring formation from chiral pool of Darabinal. By peracetylation D-arabinose changed to 2-Acetoxy-3,4-di-O-Acetylglycal, Glycosyl Bromide in presence of 33 percentage weightage of HBr/acetic acid mixture, then dehydrogenated by 1,8, di-azabicyclo undec-7-ene (DBU). Through a facial-diastereoselective

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glycosylation reaction dihydropyranone then formed from glycal in presence of a promoter $BF_3{\cdot}Et_2O$ with the use of benzyl alcohol, then ketone reduced to allyl alcohol derivative (single diastereomer) by applying Luche conditions. Then 77 % yield of cyclic hemiacetal obtained by alcohol benzylation, hydrogenolysis and alkene hydrogenation respectively. 3:7 ratio mixture of diastereomeric dimesylates obtained with use of allyl $MgBr_2$ by facilitating the diols, which then mesylated. Azido mesylated then synthesized from primary mesylate by nucleophilic substitution in presence of sodium azide and then by Staudinger reduction convert to amine and subsequently cyclization occurs resulting in 3:7 ratio of piperidine derivatives, respectively. After the two diastereomeric piperidines were separated by silica gel column chromatography and their configurations assigned to the two stereocenters, a diastereomeric mixture of azido acetates was created by substituting cesium acetate for the stereocentre of the secondary mesylate carbon in the azido mesylate mixture. This led to the production of azido alcohols and azido mesylates, which underwent one pot reductive cyclization and produced a 7:3 ratio of piperidine derivatives, respectively. Single crystal XRD analysis verified the structure through the crystallization of the appropriate HCl salt where L-arabinose was converted to glycal ent-5 and then changed again into the dibenzylpyranose derivative ent-8 as a single enantiomer, exactly like in the synthesis of 13a and 13b and the diastereomeric piperidine compounds ent-13a and ent-13b were produced by further processes employing ent-8. In the next step was to obtain all the stereoisomers of Febr. and HaF enantiomerically from pure piperidine. The free NH protected by using Cbz-Cl, alkene group conversion occur into the corresponding methyl ketone derivatives by using Wacker oxidation which then by using TMSOTf transformed to the silyl enol ether and DIPEA, then bromination with NBS produced the $\alpha$-bromo ketone respectively, undergo substitution with quinazolinone and protected (+)-isofebrifugine and (+)-febrifugine formed, eventually (+)- isofebrifugine and (+)-febrifugine gained as their di-HCl acid salt, Cbz-group undergo deprotection, the benzyl, started with refluxing the compounds in aqueous 6N hydrochloric acid, respectively. In other reaction the $\alpha$- bromo ketones substituted with 7-Br-6-Cl-4-OH-quinazoline resulting in (+)-HaF derivatives, then undergo refluxing in water with 6N-HBr acid for removal of the protecting groups, (+)- iso- HaF and (+)-HaF as their di-HBr salts obtained finally. (+)- Febrifugine and (+)-HaF and their respective diastereomers are synthesised **(Figure 4)** [86].

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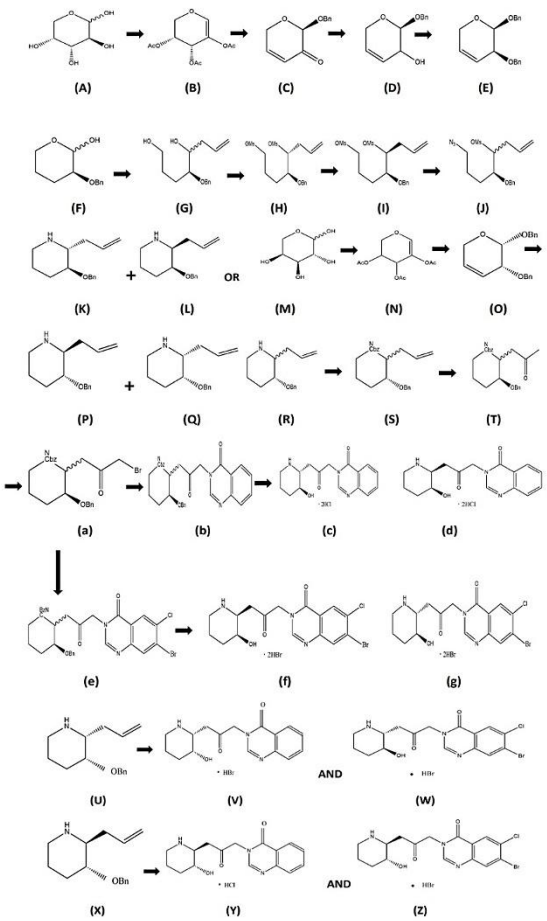


**Figure 4. Synthesis of (-)-Febrifugine and (-)-Halofuginone along with Diasterioisomers: (A)** D-Arabinose; **(B)** 5(44%); **(C)** 6(79%); **(D)** 7(95%); **(E)** 8(91%); **(F)** 9(77%) **(G)** 10a;10b(69%); **(H)** 11a, I-11b, **(J)** 12a;12b(64%); **(K)** 13a— **(L)** 13b(13a:13b=3:7 -91%); **(M)** L-Arabinose, **(N)** ent-5; **(O)** ent-8; **(P)** ent-13b **(Q)** ent-13a; R-13a;13b **(S)** 16a;16b; **(T)** 17a;17b, **(a)** 18a;18b; **(b)** 19a;19b; **(c)** (+)-Isofebrifugine.2HCl(3.2HCl- 40%); **(d)** (+)-Febrifugine.2HCl(1.2HCl-50%); **(e)** 20a;20b, **(f)** (+)-IsoHalofuginone. 2HBr (4.2HBr- 45%); **(g)** (+)-Halofuginone.2HBr(2.2HBr-51%) **(U)** ent-13a; **(V)** (-)-Isofebrifugine. HCl (ent-3); **(W)** (-)-IsoHalofuginone.HBr (ent-4); **(X)** (-)-ent-13b; **(Y)** (-) Febrifugine.HCl (ent-1); **(Z)** (-)-Halofuginone -2HBr [86].

Identification of bioactive molecules and their SAR studies are critical steps to get deep insights. A huge reservoir of new chemical entities obtained from the natural products, essentially plant sources, by NDD having diverse characteristic with more efficacy [87]. Gained through drug-discovery methods including bioinformatics, HTS, computer-modelling, chemical genetics overcoming providence and HaF was example of one of the bioactive molecules [88]. Its historical background and diverse pharmacodynamics both in vivo/in vitro models were already discussed, with molecular structure $C_{16}H_{21}O_3N_3$, named as dichroin B after the plant origin 'Dichroa', most effective among several plant extracts with antimalarial actions, in poultry feed, and *P. gallinaceum* infection in chicks, and later renamed as Febr. [89,90]. Febr. structure, used as a lead compound in isofebrifugine synthesis with less toxicity, high efficacy chloroquine-sensitive and-resistant Plasmodium species (*falciparum, vivax*) and against Plasmodium species (*P. lophurae*, *P. gallinaceum*, *P. cynomolgi*) showed better activity, overcome GIT toxicity, including diarrhoea, vomiting and liver toxicity [41,45,50,89].

Quinazoline ring SAR study shows, Br substitution conserved the antimalarial property and depressed cytotoxicity to the host cells by replacement of vulnerable protons with a Br and Cl atom and for improving Febr.'s activity, HaF was prepared ultimately [33]. In vitro, HaF-Br salt was effective against *Cryptosporidium parvum* with toxicity of racemic mixture and limits the effective dose

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range therefore trans-enantiomers (2R,3S) -(+) more efficacious, in-vivo toxicity was more in mice than its optical antipode ((2S,3R) and (2S,3R) (−) of lactate salt, with broad therapeutic window [72,90,91]. Based on the protein constituents and analogues cellular actions, smaller halogen groups like Br and Cl at R2 and R3 positions **(Figure 5)** of 4-Quinazolinone group has positive impact on potency (64 fold increase that is IC50; 0.18 μM, Kd; 30.3 nM), because in Hs-Prolyl-RS enzymes hydrophobic region for halogen atoms attachment in Sa-Prolyl-RS was minor and hydrogen or 'F' substitution reduce activity, elimination of the 'OH' of HaF enhanced antibacterial activity where single or dual substitutions with dioxole groups, $-CH_3$, $-OCH_3$, $-CF_3$, $-C(CH_3)_3$, and benzene ring showed reduction in efficacy and replacement of benzene ring with a 'N' in heterocyclic ring; compounds became inactive [92,93]. Compound with Fl and Cl substitution has strong in vitro activity against bacterial ProRS with minimum inhibitory amount, 1−4μg/mL and better membrane permeability, with more hydrophobicity than that of HaF [94].

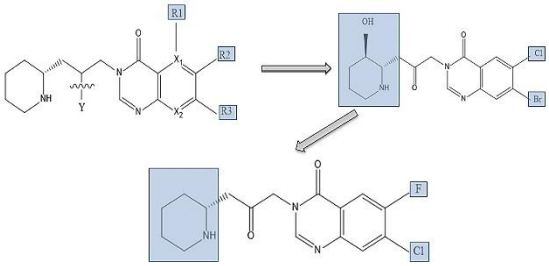


**Figure 5.** Structures of Halofuginone Analog Br and Cl at R2 and R3 regions of the 4-Quinazolinone group increased activity, H or Fl substitution at same positions reduced the activity, elimination of the 'OH' of HAF improved antibacterial efficacy where other substitutions(single or dual) with 'dioxole groups, $-CH_3$, $-OCH_3$, $-CF_3$, $-C(CH_3)_3$', benzene, showed reduced efficacies, Replacement of benzene ring with a 'N' in heterocyclic ring compound become inactive, Compound with Fl and Cl substitution has strong activity against bacterial ProRS and better membrane permeability, with more hydrophobicity than that of H [92–97].

Salt forms of HaF has benefits and toxicity as well, includes HaF-HBr ($C_{16}H_{18}BrClN_3O_3Br$) and lactate salt for coccidiosis infection in sheep, calves, hen and turkeys [98–101]. Halogen salt with high electronegativity due to less lipophilicity more polarity, and electrostatic hydrophilicity, compounds have lesser drug distribution toward BBB because of difficulty to cross lipid-bilayer membranes. Order of decreasing electronegativity from F, Cl, Br and least of I, have impact on PK and PD aspects of HaF. Drug should have amphiphilic property to dissolve, distribute and produce pharmacological action. Interaction with amino acid of various halogens vary such as 'Cl' for leucine, 'F' for glycine, 'Br' for arginine and 'I' for lysine and for cysteine, Cl, Br and I had the lowest for proline, 'Fl' had a lowest and equal tendencies for the those including 'N' and 'O' atoms, as for all the halogens highest tendency shifted towards the hydrophobic residues, when side chain interactions were measured with different geometries [100,101]. Since halogens are unique elements that have a significant impact on the structure and properties are safer for more rational drug design, it is crucial to have a thorough understanding of the non-covalent molecular interactions with pharmaceutical compounds, amino acids, and nucleic acids. These interactions involve short-range electrostatic forces due to their polar nature, which is involved in charge movements, hydrogen bonding with donors along with halogenation with proteins or nucleic acids, and carbonyl groups, are all important parameters in the structural toxicity relationships and meaningful surface area ratios assessment [102–104].

By fluorinating 3-(3-(piperidin1-yl)-propyl)-indole and piperazinyl derivatives, increase in the metabolic stability occurred by improving receptors binding, ionic channel, or total target protein sites, and also effect on lipophilicity. However, this process can occasionally have unintended effects, such as toxicity. Other effects include increasing oral absorption, blocking compound oxidation, increasing bio-availability, and increasing binding for the 5-HT-2A receptor by fluorinating 3-(4-piperidin-3-yl)-2-phenyl-indoles at the 6-C on indole [105,106]. If the substance binds to off- target

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with increased affinity it will result into toxicity such as ion channel inhibitors, anti-cholesterol, antibiotics and anti-cancerous drugs [107,108]. Halogens enhance desirable effects including stability, shelf life, intestinal permeation and BBB permeability, as a vital part of pharmacophore of the newer neuroleptic drugs. Among them 'F' is the most frequently used, depending upon interactive compound and drugs used to treat RTIs contain 'Br', 'chlorine' in antibiotics such as chlorinated form of vancomycin or cefaclor and 'I' used to treat hormonal imbalance, Canc. subtypes, in imaging and structural studies, beneficial effect on affinity for oestrogen receptors by halogenation of oestradiol analogues in sequence of; Br >Cl > I, utilized for SPECT imaging in breast Canc. diagnosis and compounds, and 3-fluorotyrosyl green used as probes, check intracellular calcium concentration alterations. [109–111]. Halogenation is the process by which peptides lose some of their conformational stability. For instance, when a helical coiled peptide's hydrophobic surface contains leucine instead of trifluoroleucine or hexafluoroleucine, the compounds' thermal and structural stability is significantly increased [112,113].

The broad-spectrum activity of HaF has been reported, includes anti-fibrosis, anti-tumor and anti-coccidia(3mg/kg in animal feed), leishmaniasis, and toxoplasmosis as well as to inhibit the growth and proliferation of Canc. cells in vitro, reduce tumor metastasis, angiogenesis in rats, and Hep.ca in mice. and growth-promoting activities [12–20,114–120]. Primary source agents of active pharmacological potential are plant origin with less toxicity and higher efficacy, playing significant role in Canc., to avoid MDR and side effect of current chemotherapeutics. HaF, is a novel anticancer drug with multiple molecular mechanism of actions and target broad range of diseases [121].

### *2.3. Clinical Significance of Halofuginone- Novel tRNA Synthesis Inhibitor:*

#### 2.3.1. Antimalarial Therapy:

The alarmingly high rates of malaria-related death and morbidity in a number of developing nations, such as South America, Africa, and Asia, along with the rise of drug-resistant parasite strains highlight the need for innovative treatments that target parasite's life-cycle. Febr. and the equivalents HaF directly secures onto two components of human Prolyl-RS, one of which mimics the bound amino acid (proline) and 3'-terminal region of tRNA, inhibiting prolyl-transfer-RNA-synthetase (Prolyl-RS) non-competitively by utilizing energy in form of Adenosine-TP. This causes an intracellular build-up of uncharged tRNA and reduces the availability of proline-containing proteins in the blood stage of the cell, as observed in P. falciparum, the asexual blood stage, and was resident in the parasite's cytoplasm [93,122]. The stage of the Plasmodium 's life cycle with asymptomatic liver, HaF targets the sporozoite in Hepatic-G-2 cells in infected mice at 17nM concentration and highly effective in in-vitro Plasmodium species (falciparum, berghei) growth retardation with equivalent speed on all three stages [123,124].

To target eukaryotic Prolyl-RS and other Amino-ARS members with greater specificity, several HaF analogues were created [125, 126]. Targeting prolyl-tRNA synthetase (Pro-RS), the AARS member responsible for linking proline to tRNA-Pro [125,126in the eukaryotes within the cytoplasm and in the presence of ATP or its analogue adenylate cyclase AMP, its analogues and cocrystals have also been clinically evaluated for managing adult solid tumor, recurrent Kaposi sarcoma, fibrotic diseases, and SARS-CoV-2 inhibitor of the invasion, infection, and replication. This mechanism is exclusive to all Amino-ARS inhibitors known to date [127–129].

#### 2.3.2. Antiprotozoal:

Orally administered HaF lactate, used as antiprotozoal for prevention of cryptosporidiosis in poultry flocks, cattle, calves and lambs, effective against Eimeria species (mitis) used in poultry feed for coccidiosis, treat lesions of different severity levels in gut, weight loss, diarrhoea or faecal oocyst counts, and decrease mortality rate. In young broiler chickens, by blocking parasite's entry into cecum of animal in the early stages, hinders parasite growth by disruption of schizonts and thereby hindering the division of the resulting unusual schizonts into schizoites, which are then eventually

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attacked by immune response [59, 130-132]. It also significantly increased body weight, reduced oocyst shedding, and prevented [133]second generation schizonts and *E. tenella* infections from destroying cecum by preventing the parasites from invading the hypothetical host cecal cell. HaF inhibits prolyl-tRNA synthetase in accordance wit[59,130–132h the codes of genetics and inhibits the production of proline-containing proteins and also catalyzes the connection of the relevant amino acid to the homologous tRNA molecule engaged in the aminoacylation reaction [78,124,134].

2.3.3. Antifibrotic Agent:

Chronic inflammation result in tissue fibrosis induced due to high concentration of transcription-growth factor-beta, Matrix-MPs, and metallo-proteinases which inhibit tissues, which regulate Extra cellular matrix (Extra-CM) turnover, destroy organ structure, function and death occur due to high levels of Extra-CM proteins, myofibroblasts, primary Extra-CM -producing cells and collagen type I. HaF, by inhibiting ProRS enzyme reduce collagen $\alpha$1-gene appearance, inhibit TGFβ-induced collagen formation by blocking Smad3 phosphorylation, and reduce levels of $\alpha$-smooth muscle positive cells [33,135–138]. Effective in Duchenne Muscular Dystrophy (Duchene-MD), pulmonary fibrosis, adhesions at various sites, hepatic and pancreatic fibrosis and chronic graft-versus-host patients, tight skin and effective in humans skin fibroblasts due to scleroderma [139–146].

HaF downstream Transcription-GFβ-Smad3 protein pathway in in vitro animal models by inhibiting prolyl-tRNA synthetase, activate Amino-AR response, inhibit T-helper-17 differentiation and concentration dependent Transcription-GFβ mediated Smad 2/3- Smad 4 complex phosphorylation and due to activation of Akt-MAP-kinase/ER-kinase and p38- MAP-Kinase phosphorylation pathway, thereby raised expression of the inhibitory Smad7 in fibroblasts, tumor cells, myoblasts, hepatic and pancreatic stellate cells, inhibit inflammatory reactions by blocking transition of fibroblasts to myofibrob lasts [33,64,135,136,138,145,147–150]. Additionally, the loss of normal living cell population (CD4+ T cells) impairs organ functions, inhibits pathogenesis, prevents tumor development in stomach carcinoma in relation to T-helper-17 cell division, and prevents con-canavali-A related fibrosis of hepatic cells by directly impacting T-helper-17 cell proliferation and destroying the tissue microenvironment [149,151–156].

2.3.4. Halofuginone Targeting metabolic Disorders:

HaF also found to be effective in various metabolic disorders due to antifibrosis activity. HaF diminishes hypoxia-induced pulmonary vascular remodelling, and pulmonary vasoconstriction, and also reverses pulmonary hypertension or contraction in pulmonary artery hypertensive patients. Also, it is a potent vasodilator of smooth muscle in pulmonary artery by triggering potassium channels, increases ions currents and by inhibiting the PI3-Kinase/AKT/ mamalian TOR signalling pathway, produces anti proliferative effect and by blocking voltage-gated Ca2+ channels through inhibition of E-C coupling decreases Ca2+ influx [157–159]. HaF by blocking transcription-GF-beta, prevents the development of diabetic nephropathy by reducing the accumulation of extracellular matrix proteins and the production of type I collagen, which is involved in fibrosis. The primary mechanism involves by blocking the expression of Transcription-GF-b type-2-receptor, which in turn inhibits the overexpression of type I collagen and fibronectin and suppresses mesangial growth in mice in the kidneys. An analysis of urine 8-OHdG levels along with dihydroethidium fluorescence supported the finding that there was a reduction in oxidative stress in the glomerulus as hyperglycemia weakens antioxidative mechanisms by releasing excessive ROS, such as hydrogen peroxide and superoxide, by glycating scavenging enzymes like superoxide dismutase (SOD). By targeting Transcription-GF-b1/SMAD 2 pathway also suppresses the development of diabetic nephropathy by halting Extra-CM deposition and decreasing oxidative stress, as diabetic nephropathy is affecting about one-third of all diabetic patients leading to end-stage renal failure, this drug could be a wonder [160].

2.3.5. Antiinflammation and Immunomodulation:

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InL -17 stimulates release of other cytokines, interleukins (InL- 17A,7F,21,22,23) and has pro-inflammatory functions, involve in formation of new blood cell, thus it directly impacts on epithelium cells, endothelial, and fibroblasts production in addition to releasing chemokines and prostaglandins, also overexpressed in rheumatoid arthritis, and SLE- individuals [161]. HaF inhibits inflammatory pathogenesis and contributes to control of autoimmune and inflammatory diseases including cancer (metstasis, angiogenesis, apoptosis induction, invasion) by directly inhibiting the Transcription-GFβ-SMAD pathway and a malfunctioning CD4-T-helper-cells associated with secretion of InL-17 [155,162–165]. Also, by targeting BLK in myeloid derived suppressed cells, inhibit pathogenesis in SLE by inhibiting the nuclear translocation of p65/p50 heterodimer resulting in increasing the mRNA expression of Blk, induced suppression of the downstream E-RK signalling pathway in order to increase the Apo. of myeloid derived suppressed cells, alleviated the disease progression, indicating potent immunotherapeutic potential by targeting prolyl-transfer-RNA synthetase and amino acid starvation response [166–169]. The anticancer effects are attributed to their inhibition of STMN-1 and p53, as well as their impact on migration, mutation, metastasis, invasion, MDR, and autophagy activation, all of these promotes early-phase cell death [170–173].

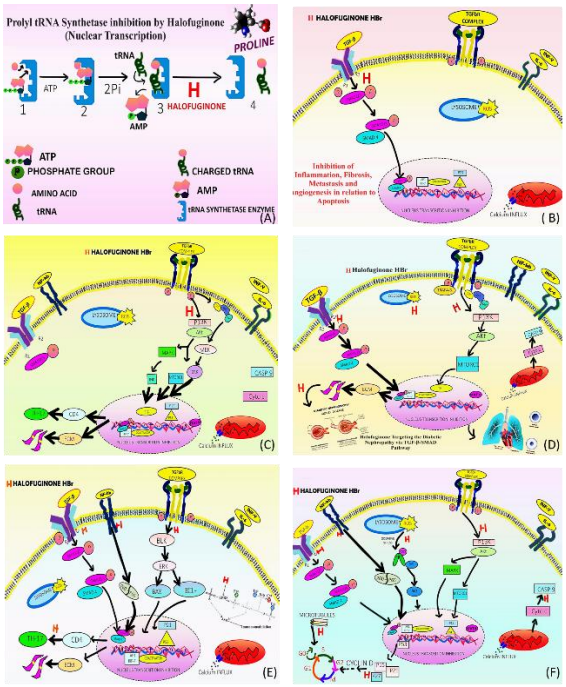


**Figure 6.** Halofuginone Targeting numerous pathways By inhibiting Prolyl tRNA Synthetase enzyme to prevent collagen synthesis in fibrosis, inflammation and to treat various diseases **(A)** Inhibiting Prolyl tRNA Synthetase; **(B)** As antifibrotic by inhibiting transcription growth factor beta (TGFβ)-induced collagen synthesis-TGFβ-SMAD3 pathway, reduce collagen $\alpha$1(I) gene expression, blocking small mother against decapentaplegic -3 (Smad3) protein phosphorylation and conversion to excitatory Smad 2/3, and co Smad4, subsequently reduce levels of $\alpha$-smooth muscle positive cells hence inhibiting transcription in nucleus and translation process in cytoplasm to collagen protein formation which is involve in fibrosis; **(C)** By targeting P13-AKT-MTOR pathways, TGFβ- Smad3 protein pathway in in vitro animal models, activate AAR response, inhibit T-helper-17 differentiation and concentration dependent TGFβ mediated Smad 2/3- Smad 4 complex phosphorylation and due to initiation of Akt-MAP-Kinase/ER-Kinase and p38- MAP-Kinase phosphorylation pathway where Akt is protein kinase-B, MAP-Kinase is mitogen activated protein kinase and ER-Kinase extracellular signal regulation; and raised amount of the inhibitory Smad7 in fibroblasts, tumor cells, myoblasts, hepatic and pancreatic stellate cells, block inflammatory and autoimmune harmful reactions by blocking transition (fibroblasts to myofibroblasts); **(D)** By blocking TGFβ-SMAD3 AND Extra-CM Pathway to inhibit Diabetic Nephropathy progression and pulmonary hypertension, reduce collagen $\alpha$1(I) gene expression, inhibit TGFβ-induced collagen synthesis by blocking Smad3 hence inhibiting transcription and translation to collagen protein formation which

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is involve in diabetic nephropathy. As a potent vasodilator effective for pulmonary hypertension treatment of smooth muscle cells in pulmonary artery by activation of K+ channels, increases voltage gated $K^+$-ions currents and by inhibiting the PI3K/AKT/mTOR signalling pathway, produces anti proliferative effect and by blocking voltage-gated Ca2+ channels through inhibition of E-C coupling decreases Ca2+ influx. Where Mammalian-TORC1 is mammalian target of rapamycin complex-1; **(E)** By inhibiting TGFβ-SMAD and T-helper-17 (TH-17) pathways to combat inflammation during autoimmune diseases and for immunomodulation reduce collagen $\alpha$1(I) gene expression, inhibit TGFβ-induced collagen synthesis, reduce levels of $\alpha$-smooth muscle positive cells hence inhibiting transcription and translation to collagen protein formation. By directly inhibiting a dysregulated distinct subset of cluster of differentiation (T helper cell-CD4), T helper-17 cells with cytokine IL-17, result in inhibition of inflammatory pathogenesis, and contribute to anti-inflammatory and autoimmune disease control and by inhibiting NF-kappa-b and p38-MAP-kinase pathway (in activated T-cells) [174]. Also, by targeting BLK in myeloid derived suppressed cells, inhibit pathogenesis in SLE by inhibiting the nuclear translocation of p65/p50 heterodimer resulting in increasing the mRNA expression of gene B-lymphocyte kinase (Blk), by enhancing kinase activity of Blk via direct molecular binding and also Blk induced suppression of the downstream ERK signalling pathway in order to increase the Apo.; **(F)** In cancer To inhibit cell-cycle progression and induce apoptosis, .target at, TGFβ-SMAD3, P13-AKT-MTOR1, P13-AKT-MAPK, CASP-9, Cell Cycle-CYCLIN D, Microtubule assembly, NFK-B-P60/P65 and Lysosome ROS-NRF-2 Pathways. by inhibiting Prolyl transfer RNA synthetase enzyme reduce collagen protein formation. By inhibiting T-helper-17, Interleukin-17, result in inhibition of inflammatory pathogenesis, Arrest cell cycle in various cancers including colonic cancer cells (G1/G0 phase) by increasing p21 and p27, lung cancer by upregulating p21, p27, Caspase-3, PARP, ROS, Bax/Bcl2 and Cyclin D1 downregulation, malignant mesothelioma by Cyclin D1 downregulation, hepatocellular carcinoma by upregulating of p15, p21, Caspase-3,9,8, PARP, and downregulating Mcl-1, cIAP1, breast cancer by targeting microRNA-31, ROS increase, by downregulating TGF-β, G2 and M phase seizure and destruction microtubules, acute promyelocytic leukaemia by upregulating p15, p21, and decreasing MYC, S phase arrest, cell death, colonic cancer by upregulating Caspase (3,9,8). Inducing Apo. in different cancers by targeting various pathways including erythroid related factor of nuclear factor-2 NRF2, and Phosphatidylinositol 3 -kinase (PI3-Kinase)/Akt signalling pathway [175].

### *2.4. Halofuginone Targeting Various Cancer Subtypes:*

#### 2.4.1. Gastric Cancer and Colorectal Cancer:

According to the GLOBOCAN statistics report in 2020, 5th most common cancer(Canc.) globally is gastric (GC), and the 4th major cause of Canc. associated mortality with more than 1M morbidity rate. Annually >1.2 M colorectal malignancy cases are also reported, involving majorly lower GIT, with more than 600K deaths rate globally and 8% mortality rate in United States, 2nd leading cause of death, with main mode of action is induced by preliminary genetic alteration, environmental factors, and lifestyles habits and A-kt-serine/threonine kinase B pathway overexpression involve in Canc. cell proliferation, glucose metabolism, transcription and cell metastasis [176–178].

Because of chemoresistance, the current available chemotherapy treatments are ineffective. GC sometime secondary to *H. Pylori* infection and associated with gastric-perforations and involving the stroma of gastric tumor Canc.-associated fibroblasts causing a huge risk, and also poor prognosis. There are limited available therapies, with main molecular target includes Signal-TAT-3 (involve in changeover of signals from receptors for several growth factors), VEG-Factor and membrane receptors of the cytokines- InL-6 family. In addition to targeting the Janus kinase/STAT route and the Extra-CM elements secreted and transformed by CAFs in stomach Canc. HaF inhibits the phosphorylation of Signal-TAT3 and the ensuing pathway, which in turn inhibits the cytotoxicity of tumor cells, results in decreased size of the tumor by preventing connections between Canc. cells and the stromal space, fibrosis and microscopic vessel density in human tumor xenografts in mice, and blockade of VEG-Factor signalling, which in turn inhibits tumor metastasis[179–181].

In colorectal Canc. PI3-Kinase/A-kt/m-TOR signalling activated through phosphorylation, signalling downstream factors, follows by regulation of cellular process in Canc. cells and regulate

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mammalian-target of rapamycin complex-1 through phosphorylation at Ser-2448, hence this pathway along with high-grade intraepithelial neoplasia overexpressed in Canc. cell of colon majorly involving glands (carcinoma) [182–184]. Also colon mutation involves glucose breakdown to pentose-phosphate (pathways for NADPH) in malignant cells was regulated by p70-S6K synthesis of lipid/sterol involve in glucose metabolism, producing redox state and promotes Canc. proliferation, and via reactive oxygen compounds (ROS), the pentose-phosphate-pathway enables Canc. cells escape death. Another well-studied m-TORC-1 downstream factors include 4EBP1, which deactivates the eukaryotic translation initiation factor 4E(eIF4E) [185,186]. HaF has shown the anticancer activity both outside body and in vivo in colorectal Canc. by the suppression of A-kt/ m-TOR-C-1 signalling and glucose metabolism along with lipid profiles by constraining G-6P-D, suppressing glucose-transporter GLU-T-1 and hexo-kinase-2 in glycolysis, glucose-derived Tri-C-Acid cycle flux in Canc. cells hence, inhibited cell growth, release ROS, mitochondrial dysfunction, reduced CRC cell viability and induced Apo.[187]. Arresting cell cycle, in the G1/G0 phase of the Colorectal Canc. cells, causing a reduction in Nictoniamide-AD-PH as a result of the deactivation of glucose-6P-dehydrogenase. Causing Canc. cell death by increasing the amount of Cas-3 and separated P-ARP due to the generation of ROS [188–192].

2.4.2. Breast Cancer:

Breast Canc. (B.Canc.) one of the commonest sybtype worldwide in 2020, total of 2.3M new cases emergence, in both sexes accounts for 1 in every 8, representing a quarter of all cancerous cases in females. Principally in transitioning countries with an estimated 68.5K women mortality rate in 2020 corresponding to 16% Canc. deaths because of insufficient public health awareness. But improvement appeared due to presentation of the Global Breast Canc. Initiative (by WHO), early diagnosis and adequate therapeutic guidelines, reducing the disease global burden [6,193,194]. B.Canc. on the basis of results from immuno-histo-chemistry and *in-situ*-hybridization testing categorized as human epidermal-growth factor-receptor 2 positive (HER2) or HER2 negative as per the 2018 American Society of Clinical Oncology and National Comprehensive Canc. Network scoring guidelines, and 80% are categorized as HER2-negative, nearly 60% fall into HER2-negative category [195–198].

One of the two major complex transcription regulators is signal-transducer/activator of transcription-3, (which includes Signal-TAT-1,2,3,4,5a, 5b, and 6), mediates the transfer of signals from the membrane to the nucleus and is a necessary gene transcription component. Other one transcription regulator is erythroid derived-Nuclear factor-2, contribute in a diversity of physiological processes and overexpressed in various Canc. subtype and help in tumor progression [138,194,199,200]. In Canc., overactive Signal-TAT-3 promotes cell growth while inhibiting cell death, persistent signalling increases tumorigenesis caused by increasing apoptotic genes inhibition, majorly Bcl-2, Mcl-1, and cyclins cell cycle regulatory including D1 or D2, and downregulating of endogenous of negative regulators [199–202]. Excessive activation of Nuclear-F-2 in malignant cells facilitates survival by activating a non-canonical pathway and interacts with Signal-TAT-3 signalling pathways, and influences Canc. progression [175,203,204].According to another study, Signal-TAT-3 boosted Nuclear-F-2 expression as a result of interleukin (InL) -6 secreted by stellate cells in pancreas, which caused Extracellular-MT traits and cause metastasis [205]. Signal-TAT-3 and Nuclear-F2 complex form, binds to the InL -23A gene promoter region, produces protein, which bind to InL-23A receptors in BL- B.Canc. - cells, signals for cell division [205].

HaF suppress Nuclear-F-2 and Signal-TAT3 viable B.Canc. cells, impede the progression. As both Nuclear-F-2 and Signal-TAT-3 higher level manifested in B.Canc., fast development of Canc. occur because of InL-23-A release, a common proinflammatory cytokine expression induction, because Nuclear-2 form a complex with Signal-TAT-3 (Y705 phosphorylated dimeric type) with worse survival rate of patients resulting in tumor growth and metastasis including B.Canc.[205,206].

2.4.3. Lung Cancer:

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Almost highest morbidity and mortality rate are associated with lung-derived Canc., and difficult to treat, in both sexes worldwide, sub categorized as non-small-lung-Canc., meso-thelioma arises from the malignant transformation in pleural cells, pericardial cells, or tunica-vaginalis [207,208]. Main molecular mode of action is Mitogen activation of Epithelial-GF-R which by phosphorylation, activate several cancerous signalling pathways including Signal-TAT-3, PI-3-Kinase/Akt/m-TOR-1 and RAS/RAF/ME-Kinase/ER-Kinase1/2 involved in disrupted cell growth and survival. JN-Kinase and p38-MAP-Kinase pathways activate in response to chemical stresses, induce immune response, and cell death [209].

HaF inhibit lung Canc. by inhibiting various pathways including Transcription-GF-β signalling pathways, ER-Kinase-1/2, JN-Kinase and p-38-MAP-Kinase pathways, PI3-Kinase/AKT, NF-κB signal pathway and Signal-TAT-3, it has suppressed cancerous growth, metastasis, cell proliferation and induce apoptosis (Apo.) [148,210–213]. Stimulates apoptotic cascade in lung-derived Canc. cells by increasing the Bax : Bcl-2 ratio, activating cas-3 with concomitant Poly-AR-P cleavage, and blocking the activation of Signal-TAT-3, a downstream effector of Epithelial-GF-R and ER-Kinase1/2 and pathways. This is done via p-38/MAP-Kinase pathway [214]. HaF produces a dose-related decrease in the lifespan of cells, produce selective G1/S cell cycle arrest in cancerous versus non-malignant cell types [215].

2.4.4. Hepatocarcinoma:

One of the most commonly diagnosed Canc. worldwide is liver Canc. maybe secondary to chronic liver disease (CLDs) with estimated 830K morbidity rate in the year 2020 as per IARC GLOBOCAN Canc. statistics and ranks at the second of all malignancy-related mortality of liver Canc., in China this is the most common type of carcinoma, accounting for approximately 90% of the total, induces persisting fibrotic tissues formation/ liver destruction, result in cirrhosis development ultimately damaging the normal liver structure, recruits inflammatory cells after tissue injury and activation of hepatic-stellate cells [216–218]. Liver is responsible for all kinds of significant metabolic functions; thus, liver Canc. certainly causes greater troubles, and its treatment is extremely problematic as it has rich blood supply and close association with other digestive organs. For patients with advanced inoperable liver Canc., no current treatment as such available therefore researchers are working on plant extracts to find novel treatment, among them one is HaF with significant anti-tumor effect [32,219].

One of the studies reported that, therapeutic promising drug HaF has beneficial effects in fibrotic disease, upon oral administration at dose of 10ppm, by inducing suppressive effect on liver fibrosis by decreasing the levels of $\alpha$-SM-A, tissue inhibitors of metallo-proteinase-2 and Smad-3, thereby reducing the severity of liver fibrosis, immunomodulatory effects induction and production of nuclear- factor-kB. Levels of chemical mediators altered mainly, inflammatory cytokines such as TNF-$\alpha$, InL -6 and InL-1β in hyaluronic acid, procollagen III and Transcription-GF-β1 in the serum and collagen-I in the liver tissue, as Con-A injected into the tail vein resulting in in the serum AS-transferase and alanine amino-transferase levels increased [220–222]. Another study reported that HaF could inhibit growth of hepatocellular carcinoma Hep-3B cell line and Hep-G2 cell line both in vitro and in vivo with the subsequent molecular mechanism of liver Canc. suppression was; by arresting the cell cycle ( G0-G1 and S phase), by up-regulating the amount of p15 and p21 which are negative cell cycle regulatory proteins, by selective inhibition of CDK4/6 activity by p15 and while p21 is a cell cycle kinase essential for G1 and S phase. Hence HaF inhibit CDK2, CDK1 and CDK4/6 activity, p21 and tumor cell progression and induced Hep-G2 Apo.by activating caspase pathway proteins including Poly-AR-P, caspase-3, 8 and 9 level are increased, which often produce cleaved active products through the action of upstream proteases and down-regulate anti Apo. protein Mcl-1 and c-IAP1 expression in hepatocellular carcinoma cells [148,223–228]. HaF also inhibit ME-Kinase/ER-Kinase and upregulate Janus-Kinase pathways by increasing phosphorylation level in hepatocellular carcinoma cells in liver Canc. subsequently inducing tumor suppression [229,230].

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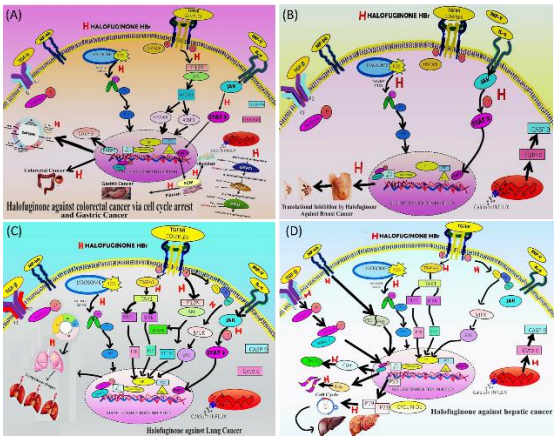


**Figure 7.** Halofuginone targeting multiple cancers; **(A)** Colorectal cancer: Inhibiting P13-AKT-MTOR, JAK-STAT, CASP-3 and Lysosome ROS-NRF-2 Pathways, inhibiting inflammation, cell cycle progression in in the G1/G0 phase, also inhibit glucose metabolism and suppress lipid profiles. Anti-cancer activity both in vitro and in vivo by suppression of Akt/ mTORC1 signalling pathway and also inhibit glucose metabolism by inhibiting G6PD, suppressing glucose transporter G-LUT-1 and hexokinase-2 in glycolysis, reduced glucose-derived T.C.A-cycle-flux mediated by Ak-t/m-TOR-C-1 signalling pathway in cancer cells hence, inhibited cell division, releases R.O.S., mitochondrial dysfunction, reduced CRC cell viability and induced apoptosis (Apo.) that is a programmed cell death with reduced level of NAD-PH due to glucose-6-PD inactivation in pentose-phosphate reaction and also in the G.1/G.0 phase of the CRC cells, a dose-dependent-arrest, inhibiting cell proliferation in cell cycle progression, and induces Apo.by increasing level of cleaved-Caspase-3 and cleaved-PARP due to ROS generation; Gastric cancer: Via targeting Janus kinase and signal transducer of activated transcription (JAK-STAT) and extracellular matrix protein Extra-CM Protein Pathways in inhibit transcription of genes involve in cancer cell cycle progression, reduce tumor volume and induce Apo.; **(B)** Breast cancer: Targeting JAK/STAT, CASP-9 and Lysosome ROS-NRF-2 Pathways, intracellular NRF2 signalling triggered because of Pancreatic-S-Cells release Interleukin-6 binds to its receptor and activation of Janus-K/Signal-TAT-3 signalling, resulting in nuclear-factor -2 (NRF-2) downstream EMT-related transcription factors inducing EMT in Panc-1 cells by inducing expression of E-MT related marker genes, to induce Apo., inhibit cell cycle progression by inhibiting growth differentiation, maturation and metastasis; **(C)** Lung cancer: Induces cell Apo., inhibit metastasis, fibrosis, growth, differentiation and inhibit inflammation cell cycle progression by targeting P13-AKT M-TOR, JAK-Signal-TAT, Cell Cycle, MEK-ER-Kinase, MK-Kinase-JN-Kinase-P-38 and Lysosome ROS-NR-F-2 Pathways, through targeting and inhibiting activation of STAT-3- a downstream effector cascade protein of endothelial growth factor receptor E-GF-R and erythroid nuclear factor ER-K1/2 and pathways and via protein 38 and mitogen-activated-protein kinase-38/MAP-Kinase induces the Apo. cascade through decreasing Bcl-2 and Bax up regulation, caspase-3 activation with Poly-ADP-RP cleavage and produces reduction in cell viability, cell retard and G1/S cell cycle-arrest cancerous cell types; **(D)** Liver cancer: Induces Apo. and suppress_cell cycle growth differentiation by inhibiting TGFβ-SMAD3, T-helper-17, CASP-9, Extra-CM, Cell Cycle progression-G0/G1 and S phase, MEK-ERK, MKK-JNK-P38, NFK-B and Lysosome ROS-NRF-2 Pathways, as antifibrotic, by inhibiting Prolyl transfer RNA synthetase enzyme reduce collagen $\alpha$1(I) gene expression, inhibit transcription growth factor beta (TGFβ)- Smad-3 protein phosphorylation and conversion to excitatory Smad 2/3, and co Smad4, subsequently collagen protein formation which is involve in fibrosis. By directly inhibiting a dysregulated distinct subset of T helper CD4 cells (T-helper-17) cells with Interleukin-17, result in inhibition of inflammatory pathogenesis, and result into autoimmune disease control by inhibiting nuclear factor-kappa-b (NF-Kb) and p38-mapk pathway in activated T-cells. Arrest cell cycle at the G-1/G-0 phase, by increasing p21 and p27, ROS, and Cyclin D1 downregulation, malignant mesothelioma by Cyclin D1 downregulation, hepatocellular carcinoma by upregulating of p15, p21, Caspase(3,9,8), and downregulating cIAP1, ROS increase, by downregulating TGF-β, phase cell cycle phases arrest at G2/M and microtubules disruption, by increasing p(15,21), and decreasing MYC, S-phase arrest, Apo., Inducing Apo. by targeting various pathways including nuclear factor -2 (NRF-2), and Phosphatidyl-inositol 3 -kinase (PI3-K)/Akt signalling pathway [231].

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*2.5. Therapeutic and Clinical Potential of Halofuginone:*

To check efficacy of HaF various trials are done both for Canc. and non-cancerous diseases in patients. Phase-I-studies of HaF to monitor the toxicities (D-LTs) and the maximum dose that can be tolerated (MTD), Phase II oral HaF studies to check systemic therapy response in patients with superficial-transitional cell-bladder carcinoma, with result showing favourable safety and tolerability profile in patients. Topically administered HaF Phase II study in patients with Acquired-I-Syndrome-related Kaposi sarcoma, serial Kaposi sarcoma biopsy assessing treatment effects on angiogenic predisposing factors and nuclear antigen-1 of Kaposi sarcoma linked with herpes-virus-latency (no effect on matrix metalloproteinase-2 (MMP-2)) and with significant heterogeneity and a decline in collagen type-I level significantly in HaF treated models [32,232]. Studies are done on solid tumor in patients, the acute Maximum Tolerated Dose=3.5milligram/day of HaF, with nausea and vomiting as Dose Limiting Toxicity, and the dose for phase II trials suggested was 0.5 mg Once-Daily. **(Table 1)** [30,233,234].

**Table 1.** Clinical trials of Halofuginone in humans.

| Disease | Clinical trials | Active ingredient | Route | Dose | Dlt | Reference |
|---|---|---|---|---|---|---|
| **Kaposi sarcoma, renal cancer. Rectum cancer, melanomas and Non-small-cell lung-cancer** | Phase II and PK | Halofuginone | Topical | 0.01% w/w ointment, twice daily | No side effect | [232] |
| **Advanced solid tumours** | Phase I and PK Studies | Halofuginone | Oral | 0.5-3.5 mg/D | Nausea, vomiting, fatigue | [235] |
| **Chronic graft vs host disease Scleroderma** | Phase I and PKStudies | Halofuginone | Topical cream | 0.1% | No side effect | [236] |
| **Covid-19** | Phase II | Halofuginone | Oral | 0.5mg, 1 mg and placebo OD for 10 days | Well tolerated | [237] |

*2.6. Effect of Halide Salt Formulations on Safety and Efficacy of Halofuginone:*

In AIDS-related Kaposi sarcoma and scleroderma trials, HaF was previously used in humans, including local application on the skin of chronic-Gversus-HD patients, which resulted in reduces collagen $\alpha$1(I) gene activity and collagen amount, as skin biopsies three months afterwards showed improvements in neck movement, without challenges and without side effects. Additionally, in solid-tumors patients, oral administration of HaF for three months significantly reduced the mean total skin score. Effective plasma levels can be reached safely of HaF tablets at doses from 0.5-3.5 mg/D and the 3.5 mg/D as maximum-tolerable dose with the side effects including excessive nausea, emesis, and tiredness and for chronic administration the suggested dose of 0.5 mg/day along with the necessity of antiemetic to control dose-limiting toxicities [32,143,232,236,237]. HaF in a SR-formulation is being examined to avoid dose-limiting-toxicities, as well as its tolerability, and PK in

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Duchene-MD individuals with OD and multiple increasing doses. HaF, combined with in-vivo-biological assays and chemistry modification approaches such as salt formations, will result in a novel and much-needed collection of drug candidates as a lead structure with greater specificity targeting various biological and pharmacological actions, improved tolerance, and efficacy.

As, we have already discussed that HBr and Lactate salts of HaF are available but possess GIT toxicity and hepatotoxicity hence not approved for use in human. HaF is basic drug with poor safety and efficacy profile in humans. Various techniques for alteration of drug physiochemical properties have previously been studied, including use of inorganic elements such as (Na+, K+), organic cations such as tromethamine, erbumine, prodrugs, salts with phosphate ion, carboxylic acids, and by the halide moiety [238–240]. Therefore, to alter the physico-chemical properties of poorly hydrophilic basic drugs, salification that is protonation by reacting acid with relevant bases, then adding the API cationic-functional groups and the anionic counterion and is one of the most attractive tools. Eventually substituted with halides contributing as a counterion up to 32% including hydrochloric, hydrobromic, and hydroiodic acids approved by orange book analysis from 1939 to 2022 over the last nine decades which showed approval of approx. six hundred novel drugs salts. The objective of salification is to modify the molecule such that the biological activity of poorly soluble active pharmaceutical ingredient with improved pharmacokinetics parameters including absorption, bioavailability, and physicochemical properties including dissociation constant, and is the most dominant, most preferred approach, practicable, ascendable method for delivering drugs but must 'not affect active ingredients safety and toxicity profile [241].

Detailed study previously showed that hydrochloric acid salts conquered among all halide counterions in the descending sequence from highest of Chlorine contributing to approx. 29% then Bromine, Fluorine and lowest of Iodine. As one study revealed that methamphetamine HCl **(Figure 8-A)** is the 1st halide salt by utilizing the halogen acids, dominates over other salts. Despite their corrosive and dangerous nature (minimized by the salification process), the development of novel drugs significantly affected by salification as it modulates the biopharmaceutical properties playing a vital role for poorly-soluble active pharmaceutical ingredients by modifying several drug characteristics [242–244]. Halide drug salts formation help in improving the solubility, dissolution, absorption, the stability of the drug (such as Pexidartinib HCl) and bioavailability for peroral administration, hygroscopicity, manufacturing and processing. Suitable for parenteral management (Cefotiam HCl) and to identify alternate formulations (SR and IR formulations of imipramine pamoate and HCl salt) [242–259]. Molecule pKa and counter-ion help in salt formation, between the drug and counterion the pKa difference (ΔpKa) decides the formation of cocrystal **(Figure 10)** [260].

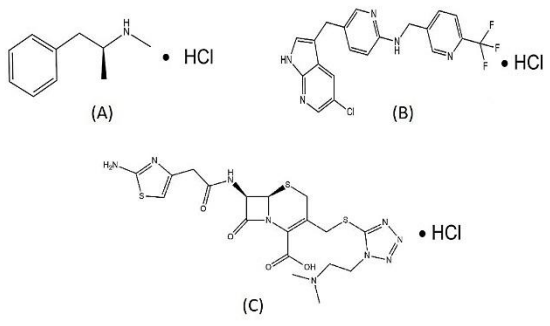


**Figure 8.** Structural examples of HCl salt containing drugs approved by FDA to elucidate importance and benefits of HCl formation of drugs for the recommendation of Halofuginone HCl salt formation in future: **(A),** Methamphetamine HCl; **(B),** Pexidartinib HCl; **(C),** Cefotiam HCl.

Afterwards methamphetamine salts, iodide salts are rare approx. 0.2% (echothiopate iodide). In last 90yrs hydrochlorides account for major drug salt form available. The FDA database indicates that by creating salt screening techniques, the use of strong acids as anions may be reduced, however it is difficult to avoid strong acids as a counterion, but precise control over the stoichiometric proportion

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of acid and base will prevent acid hazards. There have been about 171 HCl salts currently in use. According to earlier research, medications with at least one heterocyclic structure, such as amine-N-group-containing drugs like Prazosin HCl (antihypertensive), Hydralazine HCl salt (antihypertensive), and tripelenamine (an antihistaminic agent) that is absorbed quickly from the GIT are available as HCl salts **(Figure 9)** [242–244,261].

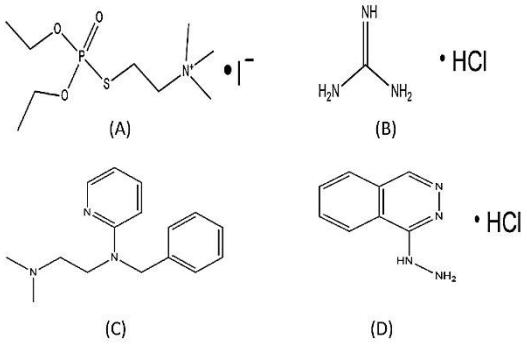


**Figure 9.** Structural examples of Halogen Containing Drugs Approved by FDA to elucidate importance and benefits of Halogen salt formation of drugs for recommendation of Halofuginone HCl salt in future: (A)-Echothiopate Iodide; (B)- Guanidine HCl; (C)- Tripelennamine; (D)-Hydralazine HCl.

Similarly previous studies shows the importance of hydrochloride salts as FDA approved hydrochloride salt of phenothiazine in 1952, has better hydrophilicity, Trifluprom azine HCl salt antipsychotic drug, free base of thioridazine hydrochloride salt (clinically used since 1962) with improve hydrophilicity, Fluphenazine HCl with better physicochemical and biopharmaceutical properties (antipsychotic agent), propiomazine HCl has higher solubility and in the case of the theoclate salt with longer duration of action, promethazine hydrochloride with high hydrophilicity, thus HCl salts seems to be the healthier choice [262–265]. Similarly, an organic base Quinoline has the HCl salts including, Ciprofloxacin HCl is clinically used with better solubility, without salt drug has low solubility and permeability. Mefloquine HCl with improved solubility (an antimalarial drug), and Moxifloxacin HCl an anti-infective, with improved stability [266,267]. FDA approved HCl salts of Piperazine scaffold beneficial with better solubility, present in almost 13 drugs, including Pentazocine HCl (FDA approved) opioid pain killer with highly water-soluble approx. 30 mg/mL , antipsychotic drug ziprasidone, Azelastine HCl salt is a phthalazine for allergic reactions, Flavoxate HCl (was developed and approved in 1970) muscle relaxant with improve water solubility and stability, trazodone HCl solubility improved, raloxifene HCl BCS class II drug (FDA approved) treat osteoporosis and donepezil HCl **(Figure 10)** [268–272].

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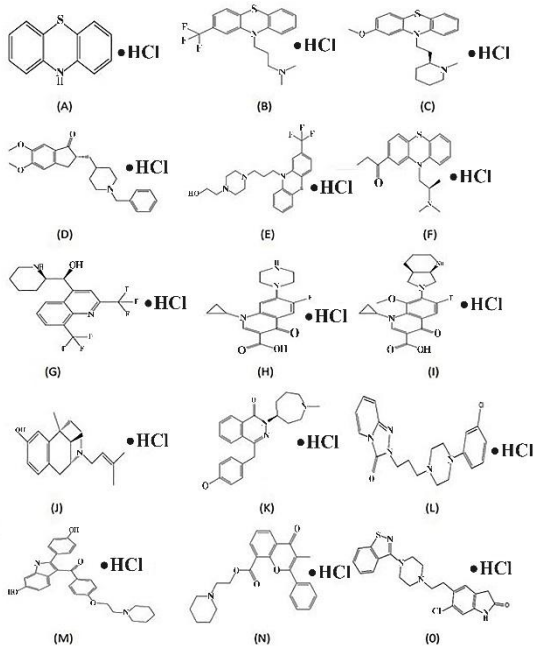


**Figure 10.** Structural examples of HCl Salt Containing Drugs Approved by FDA To elucidate importance and benefits of HCl salt formation of drugs for the recommendation of Halofuginone HCl salt in future: **(A)** Phenothiazine HCl; **(B)** Triflupromazine HCl; **(C)** Thioridazine; **(D)** Donepezil HCl; **(E)** Fluphenazine HCl; **(F)** Propiomazine HCl; **(G)** Mefloquine HCl; **(H)** Ciprofloxacin HCl **(I)** Moxifloxacin HCl; **(J)** Pentazocine HCl **(K)** Azelastine HCl **(L)** Trazodone HCl **(M)** Raloxifene HCl; **(N)** Flavoxate HCl; **(0)** Ziprasidone HCl.

Numerous FDA-approved drugs containing Chloride and bromide salts with improve solubility and stability, for drugs having quaternary nitrogen, including Pralidoxime chloride (FDA approved in 1964) and iodide (undergoes rapid deterioration) for treating organophosphorus poisoning and echothiophate iodide salt that is an organic phosphorous cholinesterase inhibitor for the treatment of glaucoma with improve water solubility of >500 mg/mL and stability [273,274]. Among the quaternary ammonium nitrogen-containing alkaloid class of drugs the bromide counterion commonly used with improve solubility and bioavailability as poorly soluble drug also cant diffuse cross blood brain barrier so salt form can transport across lipid bilayers such as HaF-HBr salt has been in use for past few decades for malaria and topically in scleroderma other examples including Tropane alkaloids, such as Hyoscine comprising tertiary nitrogen (for motion sickness) leading to the discovery of other drugs salt as well including ipratropium HBr and tiotropium HBr (for COPD and asthma as a nasal spray), Methscopolamine bromide (approved for peptic ulcers treatment), Pyridostigmine bromide (for myasthenia gravis), Galantamine hydrobromide is a tertiary alkaloid with improve stability and numerous quaternary-N-containing molecules for several indications **(Figure 11)** [275–280]. However very few drugs contain HBr salts that is why their use is reduced due to chronic toxicity of bromide ions, such as it causes gastric issues and CNS side effects including confusion and hallucinations. Similar side effects can be seen with HaF HBr [281].

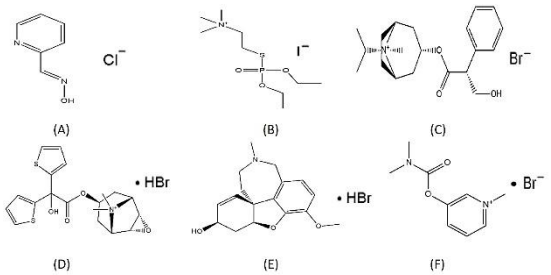

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**Figure 11.** Structural examples of drugs containing Halogens; Chlorine, Bromine, Iodine and Fluorine substitutions: **(A)** Pralidoxime Chloride; **(B)** echothiopate iodide; **(C)** Ipratropium Bromide; **(D)**Tiotropium HBr; **(E)** Galantamine HBr; **(F)** Pyridostigmine HBr.

*2.7. HCl Conjugate Formation of Halofuginone:*

Approximately 70% drugs are in salt forms documented by numerous researchers as they contain ionizable groups improving their solubility and permeability, which is related to the ionized form of molecules because they are more polar and their dissociation constant is representative of corresponding either, acidic profile (pKa less) or basic nature (pKa more) of molecules [282]. Salt formation process also employed for drug discovery and development, done by performing salt screening in order to avoid toxicity. For basic molecules such as HaF, it forms cation with the decrease in the pH and contain 'N' (enhancing 'basicity' to the molecule) and remain in ionized form, upon interaction with an anion remain in solubilized state. However, stable basic-drugs salts obtained with strong- acids due to very low pKa values such as Hydro-Cl, Hydro-Br and Hydro-I. During salt screening ΔpKa, (more than 3), ionization site for salification in basic drugs provided by nitrogen in the structure, with strong inorganic-acids (in comparison to carboxylic-acids) more stable salts obtained, and tests of halides as counterions are vital for counterion selection and are observed very closely. Hydrochloride salts possess chloride ions, are endogenous, easy to prepare, plentiful, and the most frequently occurring especially along with sodium salts. Therefore, in the future, there is need of HaF halides salt formation with hydrochloride despite halogen acids possess restrictions due to their corrosive nature, although with specially considering the drug precipitation, common ion effect, humid conditions (due to the volatile nature might be converted to mono-HCl) and pH microenvironment while selecting the drug salts **(Figure 12)**. We propose the use of HCl salts of HaF as due to the high Cl content with approx. 0.1–0.15 M it have restricted solubility or precipitate out in the gastric environment, as per conversion to free base in the stomach. And HCl addition will influence various aspects of HaF including improvement in solubility in numerous solvents, dissolution profile, absorption, formulation development, administration (in tablet, syrup, capsules, suspension parenteral) with enhanced compatibility with excipients, stability during storage in various environmental conditions, improving shelf life, effectiveness and bioavailability (as it is converted to amorphized form) and upon peroral administration, drug dose dumping occur due to high dissolution in initial stages and drug concentration surpasses its equilibrium solubility [283–286]. To overcome the risk associated, rigorous process controls may suggest a solution and ultimately help in ideal drug discovery with acceptable high standard properties.

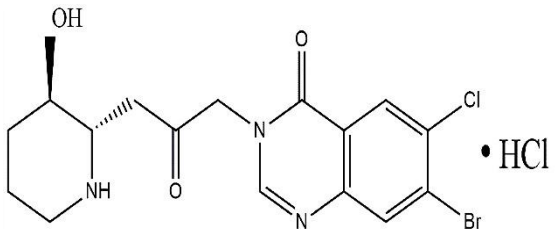


**Figure 12.** Halofuginone HCl.

*2.8. Stereoisomeric Synthesis of Halofuginone HCl Salt and Its Projected Properties:*

Through our studies we are recommending the formation of HaF-HCl Salt because it will have important and beneficial expected properties to address broad range of diseases with improved bioavailability, efficacy and less toxic adverse effects. By using Molinspiration, a Batch Property Calculation Toolkit mib, which help in molecular processing of large number of molecules at processing speed of 10,000 molecules/minute, and also in property calculation, written in Java, used in a batch mode in order to retrieve through web interface directly on our intranet. As a result, we

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obtained a calculated molecular descriptor of HaF-HCl salt. We used SMILES a line notation system for individual drugs describing chemical species structure by using short ASCII strings and associated chemicals are calculated by ChemAxon. 'O=C(C[C@H]1NCCC[C@H]1O)Cn3cnc2cc(Cl)c(Cl)cc2c3=O' is the smile for given structure of HaF with two chlorine molecules [286]. When we run it on Molinspiration bioactivity score- v2022.08, ligand energy would be for receptors GPCR, Ion channel modulator, Kinase inhibitor, nuclear receptor ligand, Protease inhibitor, Enzyme inhibitor were 0.23, 0.03, -0.09, -0.43, 0.3 and 0.36 respectively. The maximum stability is for kinase receptors and minimal for enzyme inhibitor, lower the binding energy between ligand and receptor more stable is complex, as large amount of the energy released on binding of a ligand to the protein receptor, greater will be the propensity of the ligand to associate with that protein. Molinspiration also calculate the LogP (octanol/water partition coefficient) by the very robust method as a sum of fragment-based contributions and correction factors and can practically process almost all organic and most organometallic molecules. Molecular polar surface area TPSA - a sum of fragment contributions is also calculated based on the procedure published by Ertl et al. by considering O- and N- centred polar fragments by PSA used to characterize descriptor; drug absorption, including BBB penetration, intestinal availability, bioavailability and Caco-2 permeability [287]. Molinspiration based on group contributions calculate molecule volume by fitting sum of fragment contributions to "real" 3D volume for a training set of about twelve thousand, mostly drug-like molecules, fully augmented by the semi-empirical AM1 method of 3D molecular geometries for a training set. Lipinski used "Rule of 5" for formulating properties, is set of simple molecular descriptors, named as such because of the border values are 5, 500, 2*5, and 5 and it states, that most "drug-like" molecules have number of hydrogen bond acceptors <= 10, logP <= 5, molecular weight <= 500 and hydrogen bond number of donors <= 5 and molecules not complying with any of these rules, may have bioavailability issue also. A simple topological parameter NROTB (Number of Rotatable Bonds), is a measure of molecular flexibility and descriptor of drug oral bioavailability defined as any single non-ring bond, bounded to nonterminal heavy (i.e., non-hydrogen) atom, not considering amide C-N bonds because of their high rotational energy barrier **(Figure 13)** [243,288].

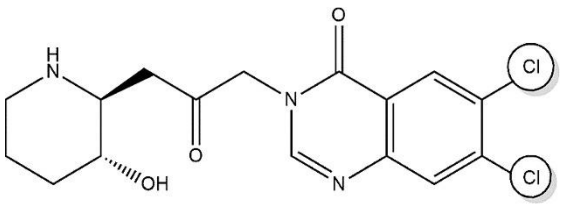


**Figure 13.** Proposed structure of Halofuginone HCl (DL-trans-7-chloro-6-chloro-3-[3-(3-hydroxy-2-piperidyl)-acetonyl]-4(3H)-quinazolinone hydrobromide).

## 3. Discussion

Among community of less than 85years of age, cancer (Canc.) is 2nd leading cause of death and each year the American Cancer Society, estimates the numbers of new Canc. cases and deaths in the United States and compiles the most recent data on population- based Canc. occurrence. In current year 2024, >20M new cases and >6M deaths estimated in U.S., but because of restriction to tobacco smoking, earlier detection and better treatment options, the Canc. mortality rate continued to decline through 2021, averting over 4 million deaths since 1991. However, during 2015 to 2019 incidence rates increased annually such as for B.Canc., pancreas, and uterine corpus Canc. by 0.6%–1%, and by 2%–3% annually for prostate, liver, kidney, and oral Canc., for melanoma, for cervical and colorectal Canc. incidence rates also increased [289,290].

It is of great significance to develop new anticancer agents with less toxicity and better efficacy. Since with the turn in century substantial progresses in outcomes for Canc. patients can be seen with the drastic variations on Canc. treatment landscape and changing trend in the approval of Canc.

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therapeutic products, with multiple molecular target and mechanism of action by FDA, currently in United States. New therapeutic approaches for Canc. with targeted drug delivery are still burning topic of interest for researchers worldwide [291], some of these trends were studied in various scientific works by researchers as one of these studies emphasize on kinase inhibitors and immune checkpoint inhibitors as the dominant product class and the second most approvals on the basis of indications but due to some limitations can't have access to the market until 2011. Others such trends include approvals for population, predicted by biomarkers and the tumour site agnostic emergence. One of the studies emphasize on these novel trends and new therapeutic approaches on Canc. treatment and their impact on future Canc. therapeutic products development with minimum toxicity [292].

The current study focused on the novel RNA synthetase inhibitor HaF which is a natural alkaloid Febr. derivative obtained from Chang Shan plant a Chinese herb, previously used in malaria and for coccidiosis. Current study guides on HaF structure activity relationship, chemical substitutions previously studied, effect in multiple organs, tissues, understanding underlying molecular mechanisms and molecular targets in various Canc. cell lines and non-cancerous diseases as it could play a vital role in novel anticancer classes of drugs and had shown positive impact but with some adverse effects which needed to be addressed. HaF by inhibiting prolyl-transfer RNA synthetase enzyme involve in proline containing protein synthesis. As we already discussed above HaF has broad scope of therapeutic activities and subsequent molecular targets, as studied in various literatures studies including antiprotozoal effect [293], anti-fibrotic studies [294], nanohydrogels as antibacterial [295–297], anti-viral such as is SARS-COV2 [298–300], anti-malarial [301], anti-inflammatory effect by targeting Nuclear-F-kB and p-38-MAP-Kinase in activated T cells, proliferation of fibroblast in rheumatoid-A disease induced by TN-Factor-alpha [36,162,226,302], immunomodulatory effect in various studies with different targets [166,303], antifungal effect [304,305], neurodegeneration protectant [306], antiarthritic effect [307], in retinopathy [308], osteoclast-genesis inhibitor [309], pulmonary hypertension [157] and cardioprotective [310] and many more therapeutic implications have been studied in which HaF has shown promising effects and used in market as an anti- coccidiosis agent in animal husbandry [311,312]. Also, its used in Covid-19 patients who are not hospitalized, has been studied in humans in phase-II clinical trials but require further evaluations [47,86,94,237,313].

Potential anticancer activity of HaF has already been studied and documented in variety of Canc. cell lines over the last decades and proven effective in a variety of Canc. both in vitro as well as in vivo multiple animal models and by targeting various molecular therapeutic targets for tumor suppression as discussed above, including for cancers of; stomach [314,315], mesoporous nanoplatform for prostate [315], bladder, pancreatic ductal adenocarcinoma [316], glioma [317], squamous cell [317,318], uterine leiomyoma [319], breast [320], nanohydrogels in canine memory carcinoma [321,322], AML [323], colorectal [324], hepatic [325], and lung adenocarcinoma [221] with combination therapies with various therapeutic agents and to overcome drug resistance to chemotherapy, HaF has shown promising effects [215,233,317,326,327].

In order to address HaF toxicity various formulations such as TPGS polymeric micelles or nano-formulations are studied but still drug is in phase 2 clinical trials in humans [328,329]. Therefore through current study we are emphasizing on preparation of novel formulation of HaF with HCl salt, as salt formation having several beneficial impacts on drug PK and PD parameters as studied by various researchers therefore before formulation development appropriate salt selection has direct impact on modifying the features of the potential target drug substance physicochemical properties with better patient compliance as it has positive effects on melting point, hygroscopicity, chemical stability, bioavailability, as well as manufacturability, aqueous solubility, dissolution rate, pH, crystallization and mechanical properties and its final properties are comparable to standard pre-formulations and should comply with the primary route of administration and dosage form. We used Molinspiration for property based virtual screening of our novel molecules parameters as it is a useful tool to pick potential drug candidates, we find out drug binding energy, Log P, and other useful and

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important properties of novel HaF with two chlorine atoms. As studied previously that HCl salt formation increased stability of API of antidepressant compound CGP6085 [330,331]. HCl salt enhance solubility of weakly basic drugs as it has a low counterion pKa, and recrystallisation from organic solvents is straightforward and is by far the most frequent choice due to simple availability and physiological reason, also form stable complex [248,332,333].

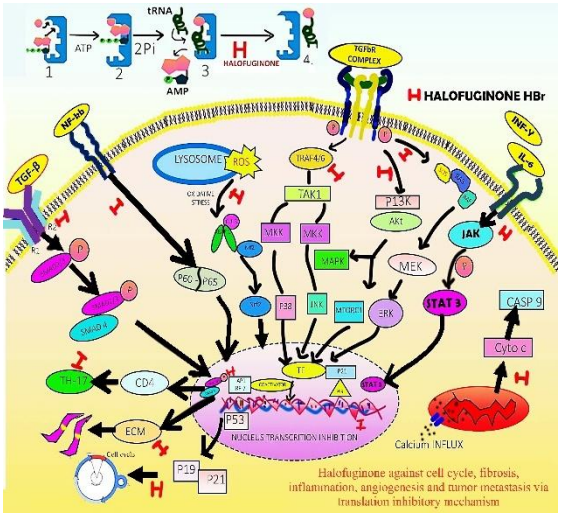


**Figure 14.** Halofuginone inhibit inflammation and fibrosis, have direct or indirect impact at various molecular levels to treat wide range of diseases by targeting TGFβ-SMAD3, T-helper-17, P13-AKT-MTOR1, P13-AKT-MAPK, CASP-9, CASP-3, Extra-CM, Cell Cycle, MEK-ERK, MKK-JNK-P38, NFK-B- P60/P65 and Lysosome ROS-NRF-2, JAK-STAT, Pathways.

## 4. Conclusions

Cancer is leading cause of mortality globally and considered as the life-threatening disease reigning champion, and for achieving operative and efficient results from cancer therapy it's important to understand the significance of early diagnosis and better novel treatment options with limited toxicity and maximum efficacy for positive subsequent outcomes on quality of life at community level. There are various natural, synthetic and semisynthetic compounds under study for novel targeted drug in therapy with limited toxicity as compared to chemotherapy induced side effects. Among these is Halofuginone, derivative of Febr. obtained from Chinese herb, which has multiple beneficial impact in various diseases due to inhibitory effect on exaggerated inflammatory immune response and also effect Treg cells, cytokines production at transcription and translation level and has various targets along with cell cycle arrest or Canc. predictive biomarkers but limited in use due to GIT toxicity and physiochemical characteristics [414]. So, in this article we emphasize on preparation of HCl salt formulation of this novel entity to minimize associated side effects and enhanced efficacy. With context to the pharmacokinetic (Absorption, Distribution, Metabolism, Elimination), pharmacodynamic and biopharmaceutical properties of Halofuginone as well. As the good choice of salt has direct impact on active pharmaceutical ingredient solubility, stability, bioavailability, therapeutic drug concentration achievement, efficacy, onset of action and other biopharmaceutical parameters, so regulatory guidelines can influence and should be considered while selecting best form of salt with maximum benefits as, well which meet safety, efficacy and quality regulatory standard as per the recommended guidelines and decision should be made according to requirement and site of administration of drug.

In order to optimize the therapeutic potential of Halofuginone, a number of important factors need to be considered for a safer and less toxic medication in the future. Thus, Halofuginone ought to be considered in subsequent research for additional assessment through modification or alternative substitutions at various positions and formulations, such as the effect of HCl salt formulation on the safety and efficacy profile of Halofuginone.

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**Author Contributions:** (AMM) data curation, primary draft; (GJK) conceptualization, project administration, supervision, final draft and funding and is corresponding author; (JB) resources, software and funding; (AAq) investigation, validation and funding; (TM) formal analysis and primary draft; (AAy) data curation, resources; (TA) review and editing; (YL) resources; (YD) review and editing.

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