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Review on analytical and bioanalytical approaches for the estimation of two anti-Covid-19 drugs

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ABSTRACT

The outbreak of the COVID-19 pandemic has resulted in a significant global mortality rate. According to the World Health Organization (WHO), the virus has impacted a population exceeding 500 million people, leading to around 6.5 million deaths. The antiviral medications remdesivir and molnupiravir are pre-existing drugs that were strategically repositioned and repurposed in the context of combating the COVID-19 pandemic. The determination of these antivirals required the establishment of rapid, sensitive, precise, and reliable methodologies. The current review entails a thorough examination of various analytical methods that have been reported for the quantitative estimation of these antiviral drugs, this includes spectrophotometric, spectrofluorometric, electrochemical, and various chromatographic techniques. Those reported techniques were applied to quantify the examined antiviral medications in a single dosage, co-formulated synthetic mixture as well as in biological fluids. Therefore, the aim of this review is to highlight the current analytical techniques for the assay of both drugs.

Keywords: *Remdesivir, Molnupiravir, Anti-viral*

1.Introduction

Coronavirus disease 2019 (COVID-19) is caused by the new coronavirus SARS-CoV-2. One of the Coronaviridae family causes fever and respiratory disorders such as dyspnea and pneumonia (Frediansyah et al., 2021). This family of viruses recombine and mutate extensively, as well as infect several species and cell types. Because of this, they evolve and re-emerge, killing many (Brian & Baric, 2005; Ziebuhr, 2005). Globally, there have been 769,806,130 confirmed cases of COVID-19, including 6,955,497 deaths, and a total of 13,499,865,692 administered vaccine, reported to WHO (*WHO Coronavirus (COVID-19) Dashboard*, n.d.). Therefore, treatment was urgent. To treat COVID-19, antiviral medicines that were previously used to treat viral infections such as hepatitis C and HIV have been repurposed. Remdesivir (REM) and molnupiravir (MLP) are two essential drugs that have been repurposed for COVID-19 treatment.

REM (**Figure 1a**) is 2-ethylbutyl (2S)-2-[[[(2R,3S,4R,5R)-5-(4-amino pyrrolo[2,1-f][1,2,4]triazin-7-yl)-5cyano3,4dihydroxyoxolanyl]methoxyphenoxy phosphoryl] amino] propanoate(*Remdesivir:DrugBank Online*, n.d.), (Fig.1 a). Gilead Sciences originally developed REM as an Ebola virus treatment (Eastman et al., 2020). It is a nucleotide analog prodrug, that hinders the viral RNA synthesis by undergoing bioactivation, transforming into GS-441524, and subsequently being phosphorylated into an active nucleoside triphosphate metabolite (Gordon et al., 2020). REM has been investigated for COVID-19 treatment against SARS-CoV-2. The FDA authorized REM as the first COVID-19 antiviral medication because it blocks virus multiplication (Lamb, 2020).

MLP (**Figure 1b**), [(2R,3S,4R,5R)-3,4-dihydroxy-5-[4-(hydroxyamino)-2-oxopyrimidin-1-yl]oxolan-2-yl]methyl 2-methylpropanoate(*Molnupiravir: DrugBank Online*, n.d.), is an isopropyl ester prodrug that is converted inside the body into the active form, β-d-N4-hydroxycytidine (Hernandez-Santiago et al., 2004). It has a broad-spectrum antiviral activity against RNA viruses like influenza, SARS, MERS, and Ebola (Reynard et al., 2015; Toots et al., 2020). Due to this, it was repurposed to be used against mild-to-moderate COVID-19 cases (Yip et al., 2022). It's a nucleoside that exerts inhibitory effects on the RNA-dependent RNA polymerase (RdRp) enzyme of the SARS-CoV-2, leading to the induction of multiple errors during the replication process of the RNA virus. It has the potential to reduce the pathogenesis and replication of coronaviruses (Pourkarim et al., 2022).

Various analytical techniques have been established for the determination of those antivirals in their pure state, pharmaceutical formulations, and biological samples. This review article provides a comprehensive overview of the available analytical techniques for determining REM and MLP in different matrices.

2. Review of the analytical methods

2.1. Spectrophotometric methods

Several spectrophotometric approaches have been reported for the quantification of REM and MLP in both bulk and pharmaceutical formulations. These techniques are outlined in **Tables 1 and 2** for REM and MLP, respectively.

2.2. Spectrofluorimetric method

A few approaches have been published for the spectrofluorimetric estimation of REM and MLP, as described in **Tables 3 and 4.**

2.3. Chromatographic methods

Many chromatographic techniques including HPLC, UPLC, and TLC have been developed and reported for the determination of REM, MLP, their active metabolites, and/or degradation products in different matrices such as pharmaceutical dosage forms and biological samples. These techniques are listed in **Tables 5 and 6.**

2.3. Electrochemical methods

2.3.1. For remdesivir

Four potentiometric sensors were applied for the determination of REM in human plasma using. Sensor I implemented Calixarene-8 (CX8) to the electrode. Sensor II had a layer of dispersed graphene nanocomposite coating. Sensor III was fabricated using nanoparticles of polyaniline (PANI) as ion-to–electron transducer. A reverse-phase polymerization using polyvinylpyrrolidone (PVP) was employed to create a graphene-polyaniline (G/PANI) nanocomposite electrode Sensor IV, A graphene-polyaniline (G/PANI) nanocomposite electrode was created applying a reverse-phase polymerization with polyvinylpyrrolidone (PVP). Sensor II and IV exhibited linear response over the concentration ranges of 10^{-7} to 10^{-2} mol/L and 10^{-7} to 10^{-3}, respectively while sensors I & III displayed linearity within 10^{-6} to 10^{-2} mol/L (El Azab & Ahmed, 2023).

2.3.2. For molnupiravir

A sensitive method based on electrochemical deposition of reduced graphene oxide (rGO) on a glassy carbon electrode (GCE) using cyclic voltammetry (CV) was developed for MLP quantitative analysis in capsule formulations, using square wave voltammetry (SWV) MLP electrochemical oxidation was examined and a well-defined peak at 0.2 V was measured against the Ag/AgCl reference electrode with the rGO sensor in Britton-Robinson buffer (BRB) pH 9. The sensor exhibited a linear range from 0.09 to 4.57 µM with a detection limit of 0.03 µM (Kablan et al., 2022).

Using cyclic voltammetry, a highly sensitive electrochemical method for quantifying MLP was reported, implementing a disposable screen-printed reduced graphene oxide 2.5% electrode (SPrGOE 2.5%), in the presence of 0.04 M BRB at pH 2 and 10^{-4} M SDS. The method was rectilinear over a range of concentrations from 50 to 6017 ng/mL. The selectivity and the sensitivity of the method were enhanced by adding the reduced graphene nanoparticles; thus, the detection limit was lowered to 15.98 ng/mL (Nabile et al., 2023).

Another sensitive voltammetric MLP determination utilizing a magnetite nanoparticle modified carbon paste electrode (Fe_3O_4@CPE) was reported. MLP was irreversibly oxidized at Fe_3O_4@CPE at 760 mV in pH 2.0 BRB by cyclic voltammetry. The excellent electrocatalytic efficiency of Fe_3O_4 NPs enhanced MLP peak current by thrice at Fe_3O_4@CPE compared to bare CPE. By differential pulse voltammetric measurements using the fabricated electrode, MLP showed a linear range of 0.25-750 µM, with detection limit down to 0.05 µM (Vural et al., 2023).

Conclusion

The current review encompasses an overview of diverse methodologies and approaches employed in the quantification of remdesivir and molnupiravir, based on analytical study reports. The review would provide valuable insights to the analytical chemist, enabling them to comprehend the fundamental solvents and their respective combinations suitable for the instruments utilized in the analytical laboratory. By examining the comparative data presented in published scientific literature, analytical chemists can acquire knowledge regarding the benefits and drawbacks of different techniques.

Disclosure

The authors report no conflicts of interest in this work.

Table 1: Reported spectrophotometric methods for the determination of REM

Matrix	Method	λmax (nm)	Ref
Pure and pharmaceutical preparation	Ion-pair complex formation with bromophenol blue	418	(Abdelazim & Ramzy, 2022)
REM in the presence of favipravir (FAV) in spiked human plasma	Method I ratio difference	Difference between (247 and 271 nm) and (222 and 256 nm) of the derived ratio spectra	(Batubara, Abdelazim, Almrasy, et al., 2023)
	Method II first derivative of ratio spectra at $\Delta\lambda = 4$ and a scaling factor of 100	At 228 and 251.2	
Standard REM and pharmaceutical dosage forms	Direct measurement of absorbance in deionized water	247	(Bulduk & Akbel, 2021)
Bulk form and pharmaceutical formulation (injection)	Charge transfer complex formation (CTC) in methanol with chloranilic acid	530	(Darwish et al., 2022)
REM and FAV single dosage forms and simultaneously in spiked human plasma	Method I direct measurement of absorbance in ethanol Method II derivative spectrophotometry Method III dual-wavelength method Method IV ratio subtraction Method V derivative ratio	244	(Elama et al., 2023)
REM and FAV in pharmaceutical form and spiked human plasma	Three chemometric based models; classical least squares, principal component regression, and partial least squares.	_____	(Imam et al., 2023)
REM and FAV Pharmaceutical formulations and laboratory prepared mixtures	Method I absorption correction method	241	(Noureldeen et al., 2023)
	Method II dual wavelength method (DW)	Absorbance difference between (244.8 and 228.8 nm) and (235.8 and 248.2 nm)	
	Method III first derivative method (1D)	237.2 and 380 nm	
	Method IV first derivative of ratio spectra method (1DD)	Using FAV as a divisor at 290 nm	

Table 2: Reported spectrophotometric methods for the determination of MLP

Matrix	Method	λ_{max} (nm)	Ref.
Pure and Capsule dosage forms	Diazo coupling with sodium nitrite in acidic solution	515	(Abdelazim et al., 2023)
Pure and tablets	Direct measurement of absorbance in methanol	236	(Deshpande & Shaikh, 2023)
MLP in the presence of its degradation products	Direct measurement of absorbance in deionized water	270	(Edrees et al., 2023)
Pure and laboratory prepared capsules	Direct measurement of absorbance in methanol	233	(M. Abdel Moneim et al., 2023)
Pure bulk powder and pharmaceutical formulation	Direct measurement of absorbance in a mixture of ethanol and water in ratio (1:1, v/v)	230	(M. S. Attia et al., 2023)
Pure and tablet dosage forms	Formation of colored ion pair complexes with thiocyanate ions	620	(R. V. Rajan & Tiwatane, 2022)
MLP in the presence of FAV in laboratory prepared co-formulated dosage form	Four multivariate chemometric models (CLS, PCR, PLS-1, and GA–PLS-1)	The selected spectral zone was from 210 to 350 nm at 1 nm interval to obtain 141 spectral points.	(Sharaf et al., 2022)
Pure and tablets	Formation of colored complexes in acidic medium, extracted in chloroform		(V. R. Rajan & Prathamesh, 2023)
	Bromophenol blue,	420	
	Solochrome dark blue	495	
	Bromocresol green	430	

Table 3: Reported spectrofluorimetric methods for the determination of REM

Matrix	Method	λex nm	λem nm	Ref
REM in the presence of apixaban (APX) in pure form and spiked human plasma.	Second order synchronous fluorimetry at Δλ = 150 nm in distilled water	Δλ = 150	REM at 410 and APX at 469	(Batubara, Abdelazim, Gamal, et al., 2023)
Formulated IV infusion and in spiked human plasma.	Native fluorescence measurements at pH 4 in water	244	405	(Elmansi et al., 2021)
REM simultaneously with simeprevir (SIM) in spiked human plasma	Zero order synchronous fluorimetry at Δλ = 90 nm in ethanol.	Δλ = 90	REM at 283 and SIM at 341	(El Sharkasy et al., 2022)
REM along with favipravir (FAV) and hydroxychloroquine (HDQ) in spiked human plasma	First order synchronous fluorimetry at Δλ = 130 nm	Δλ = 130	REM at 384, FAV at 423, and HDQ at 394	(Ramzy et al., 2022)
REM in pharmaceutical formulation and spiked human plasma	Native fluorescence measurement in distilled water	241	410	(T. Z. Attia et al., 2022)
	Micellar enhanced fluorescence using 2 % w/v sodium dodecyl sulfate (SDS)			

Table 4: Reported spectrofluorimetric methods for the determination of MLP.

Matrix	Method	λex nm	λem nm	Ref.
Pure, tablets, and real plasma samples	Fluorescence quenching of polyamine quantum dots (PA@CQDs) by MLP	440	504	(Salman et al., 2022)
Pure, capsules, and real plasma samples	Metal-chelation of MLP with 1.0 mM zinc (II) in an acetate buffer (pH 5.3)	340	386	(Salman et al., 2023)

Table 5. Reported chromatographic techniques for the determination of REM

Matrix	Stationary phase	Mobile phase	Elution mode	Detector	Ref.
Standard REM and pharmaceutical dosage forms	C18	20 mM potassium dihydrogen phosphate solution and acetonitrile (50:50, v/v)	Isocratic	UV detector at 247 nm	(Bulduk & Akbel, 2021)
REM along with oseltamivir, daclatasivir, and dexamethasone	BEH C18	methanol and ammonium acetate (8.1818 mM) in a ratio of 75.7: 24.3 (v/v)	Isocratic	PDA at 239 nm	(El-Shorbagy et al., 2023)
REM in the presence of favipravir and dexamethasone in human plasma	C18	Methanol, acetonitrile, and water acidified by orthophosphate (pH = 4) in a ratio of (15: 35: 50, by volume)	Isocratic	DAD at 240 nm	(Emam et al., 2022)
REM and its degradation product in injectable Drug product	C18	Ortho-phosphoric acid in water with pH 3.0 (A) and mixture of acetonitrile, methanol, and water in the ratio (%) 70:20:10, v/v (B)	Gradient	UV at 242 nm	(H. R. Reddy et al., 2022)
Intravenous Dosage Form	Inertsil ODS-3V	Water (acidified with o-phosphoric acid, pH 3) (A) and acetonitrile (50:50, in volumes) (B)	Isocratic	Diode array at 246 nm	(Jitta et al., 2021)
Injection dosage form	C18	Acetonitrile (A) and water (acidified with phosphoric acid, pH 4) (B) in the ratio of 55:45 (v/v)	Isocratic	Fluorescence at λex/em 245/390 nm and diode array at 240 nm	(Hamdy et al., 2021)
REM in the presence of 6 co-administered therapeutics in human plasma hydroxychloroquine, azithromycin, paracetamol, dexamethasone, rivaroxaban and edoxaban.	C18	Water (acidified with phosphoric acid, pH 4) (A) and acetonitrile (B)	Gradient	Fluorescence at λex/em 245/390 and diode array at 240 nm	(M. M. Abdel Moneim et al., 2021)
Human plasma	C18	0.05 % (v/v) formic acid in water (A) and acetonitrile (B)	Isocratic	PDA at 254 nm and MS/MS	(Pasupuleti et al., 2021)
REM and its metabolite in human plasma	HSS T3	10 mM ammonium formate in 5% methyl alcohol, pH = 2.5 (A) and methanol (B)	Gradient	MS/MS	(Xiao et al., 2021)
REM and its metabolite in human plasma	HSS T3	Phase A 0.05% formic acid in water Phase B 0.05% formic acid in acetonitrile	Gradient	MS/MS	(Avataneo et al., 2020)
REM and its metabolite, along with dexamethasone in human serum	EC-C18	Water (A) and acetonitrile (B)	Gradient	MS/MS	(Reckers et al., 2021)

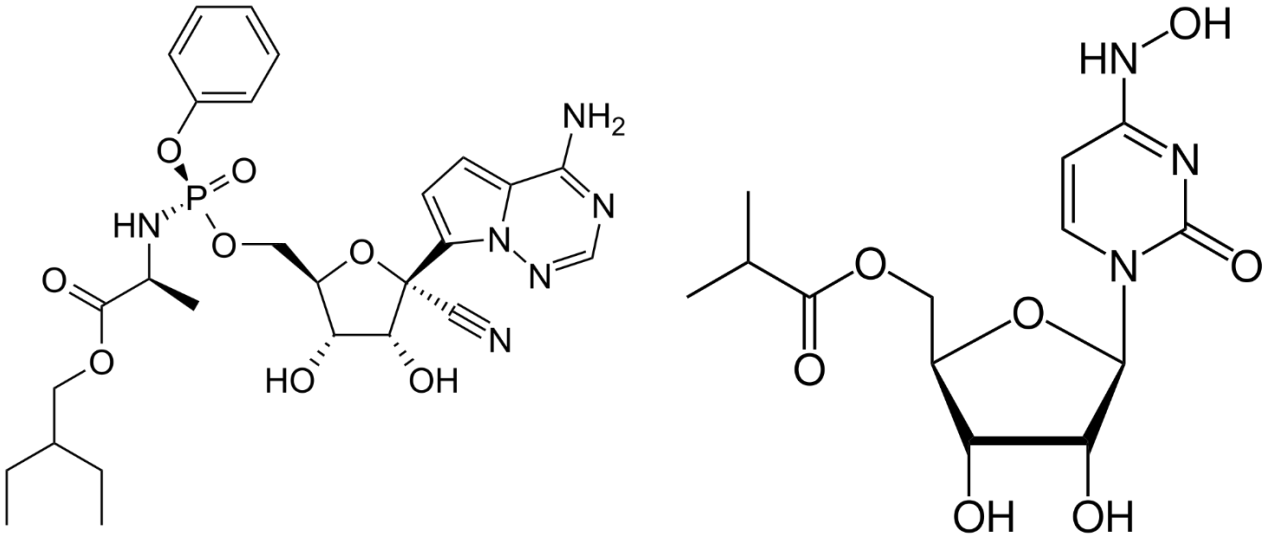
REM and its metabolite in human serum in the presence of chloroquine, hydroxychloroquine, lopinavir, ritonavir, favipiravir and azithromycin	**MassTox® TDM Master**	**Water (A) and acetonitrile-formic acid in the ratio 99.9:0.01, v/v (B)**	**Gradient**	**MS/MS**	**(Habler et al., 2021)**
REM and its metabolite in human plasma	C18	10 mM sodium formate buffer in 0.1% formic acid- water (A) and acetonitrile (B)	Gradient	MS/MS	(Alvarez et al., 2020)
REM and its active metabolite in rat plasma	C18	Mobile phase A (ACN: water = 95:5, v/v, 0.1% formic acid) and phase B (water: ACN = 99:1, v/v, 0.1% formic acid)	Gradient	MS/MS	(Du, Wang, Yang, et al., 2021)
Human plasma	Synergi™ Fusion-RP	1% formic acid in water (A) and 1% formic acid in acetonitrile (B)	Gradient	MS/MS	(Nguyen et al., 2021)
REM in addition with arachidonic acid and cascade metabolites in rat plasma	BEH C18	0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B)	Gradient	MS/MS	(Du, Wang, Zhao, et al., 2021)
REM and its metabolite in mouse tissues	BioBasic AX	Phase A: ACN-water (3:7, v/v) with 10 mM ammonium acetate (pH 6.0) Phase B: ACN-water (3:7, v/v) with 1 mM ammonium acetate (pH 10.5)	Gradient	MS/MS	(Hu et al., 2021)
REM and its active metabolite in human plasma	C18	10 mM sodium formate buffer in 0.1% formic acid (A) and acetonitrile (B) starting from 0% of (B) to 100% in 2 min and hold for 1 min to 100%.	Gradient	MS/MS	(Kumar et al., 2022)
REM along with favipravir in plasma	BEH C18	0.2% formic acid with acetonitrile (50:50)	Isocratic	MS/MS	(Harahap et al., 2023)
Bulk form and pharmaceutical formulation, and in presence of degradants	TLC silica gel aluminum plates 60 F254	Ethyl acetate and ethanol (96: 4, v/v)	_____	Densitometric detection at 245 nm	(Abo-Gharam & El-Kafrawy, 2022)
REM and FAV in pharmaceutical formulations and spiked plasma	silica gel Al plate (60 F254, 0.1 mm thickness)	Ethyl acetate–methanol-ammonia (8:2:0.2 by Volumes)	_____	Densitometric detection at 235 nm	(Noureldeen et al., 2022)

Table 6: Reported chromatographic techniques for the determination of MLP

Matrix	Column	Solvent system	Elution Mode	Detection	Ref.
MLP and its degradation products	HSS T3 C18	Ammonium formate and acetonitrile	Gradient	UV detection at 272 nm	(Jain et al., 2021)
Pure, laboratory prepared capsules and spiked human plasma	ODS -3	Acetonitrile and distilled water acidified with orthophosphoric acid (pH 3) with ratio 87:13	Isocratic	DAD at ٢٣3 nm	(M. Abdel Moneim et al., 2023)
MLP and its degradation products in bulk and pharmaceutical product	C18	٠,١ % acetic acid and ethanol (70:30 v/v)	Isocratic	DAD at 235 nm	(Bhangale & Bhandare, 2022)
Bulk powder and pharmaceutical formulation	C18	٢٠mM phosphate buffer pH 2.5 and acetonitrile (80:20, v/v%)	Isocratic	UV detection at 230 nm	(Annadi et al., 2022)
MLP raw material	C18	١٠mM Phosphate Buffer pH:7 and acetonitrile mixture (80:20)	Isocratic	DAD at 230	(Camlik et al., 2022)
Pure and tablet dosage form	C18	Methanol: phosphate buffer (25:75) (pH-3.4)	Isocratic	UV detection at 270 nm	(Suresh et al., n.d.)
Bulk and tablet dosage form	ODS C18	Methanol and phosphate buffer pH-4.2 adjusted with Orthophosphoric acid solution in the ratio of 35:65% v/v	Isocratic	UV detection at 236 nm	(Sravanthi et al., 2023)
MLP analytical standard and pharmaceutical capsules in the presence of favipravir	RP-C18	0.1 M SDS, 0.01 M Brij-35, and 0.02 M monobasic potassium phosphate mixture and adjusted to pH 3.1	Isocratic	UV det. 230 nm	(Sharaf et al., 2022)
MLP in presence of its degradation products	C18	ACN: water (20:80 v/v) mixture	Isocratic	DAD At 240 nm	(Reçber et al., 2022)
MLP and its degradation product in pure and marketed formulation	C18	Orthophopoaricacid: Acetonitrile (60:40)	Isocratic	PDA at 253 nm	(Bindu et al., 2022)
MLP and its metabolite, ß-d-N4-hydroxycytidine in human plasma and saliva	C18	1 mM Ammonium acetate in water (pH 4.3) and 1 mM Ammonium acetate in acetonitrile	Gradient	MS/MS	(Amara et al., 2021)
MLP and D6-MLP in rat plasma after oral administration	Phenyl column	Methanol and acetonitrile at a ratio of 60:40	Isocratic	MS/MS	(K. T. K. Reddy & Haque, 2022)
MLP and its genotoxic impurities in drug substance and dosage forms.	HSS T3 C18	٠,١ % formic acid in water and acetonitrile	Gradient	MS/MS	(Nakka et al., 2023)
MLP active metabolite (NHC) in pure and plasma samples	C18	Methanol0.2 % acetic acid (5:95, v/v)	Isocratic	MS/MS	(Gouda et al., 2022)

β-D-N4-hydroxycytidine (NHC) in human plasma	**C18**	**0.1% formic acid in water and 0.1% formic acid in acetonitrile for NHC in plasma**	**Gradient**	**MS/MS**	**(Parsons et al., 2021)**
β-D-N4-hydroxycytidine-triphosphate (NHCtp) in peripheral blood mononuclear cell lysates (PBMC)		50 mM ammonium formate: 5 mM ammonium hydroxide (MPA) and 80 mM ammonium formate: 8 mM ammonium hydroxide in [80:20] water: acetonitrile (MPB) for NHCtp in PBMC			
Pure and laboratory prepared capsules along with favipravir and ritonavir	Silica gel 60F254 TLC plates	Methylene chloride: ethyl acetate: methanol: 25% ammonia (6:3:4:1, v/v/v/v)	_____	Densitometric detection at 289 nm	(Saraya et al., 2022)
MLP in presence of its degradation product	Silica gel HPTLC F254 plates	Toluene: n-Butanol: Methanol: Water developing system (5:3:1.5:0.5, by volume).	_____	Densitometric detection at 276 nm	(Tekade & Patil, 2022)
Pure and laboratory prepared capsules	Silica gel-60 HPTLC plates (F254)	Methanol & glacial acetic acid (10:0.05)	_____	Densitometric detection at 233 nm	(M. Abdel Moneim et al., 2023)

Figures



a) b)

Figure 1. Chemical structure of a) remdesivir b) molnupiravir

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